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and
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Glyphosate

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

Version History

When	What
2021/06	Initial RAR

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

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B.6. TOXICOLOGY AND METABOLISM DATA

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

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

B.6.2. ACUTE TOXICITY

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

B.6.3. SHORT-TERM TOXICITY

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

B.6.7.1. Neurotoxicity studies in rodents

B.6.7.1.1. Acute neurotoxicity

Data point:	CA 5.7.1/001
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute neurotoxicity study in rats
Report No	[redacted]/P/4866
Document No	Not reported
Guidelines followed in study	No guideline stated in the report but in general compliance with OECD 424 (1997)
Deviations from current test guideline	No deviations were noted.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a

	Conclusion AGG: The study was conducted in accordance with OECD 424 and is considered to be acceptable.

Full summary

Groups of 10 male and female Alpk:APfSD rats per were administered single oral doses of 0, 500, 1000 or 2000 mg/kg bw glyphosate acid. All animals were observed daily for any changes in clinical condition for two weeks post administration. Detailed clinical observations including quantitative assessments of foot landing splay, sensory perception and muscle weakness were performed at weekly intervals. Locomotor activity was also monitored at weekly intervals. At scheduled termination 5 rats/sex/group were subjected to full *post mortem* examination. Selected nervous system tissues were examined microscopically.

Clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) occurred during Day 1 but were limited to 3 females at approximately 6 hours post treatment, in the highest dose group (2000 mg/kg bw/day). One of these females was subsequently found dead on Day 2. These clinical signs were considered to reflect general toxicity associated with the administration of high dose levels of glyphosate acid.

Slight reductions in food consumption, without any associated effects on body weight, were also observed during Week 1 for both sexes in the highest dose group.

Quantitative assessment of neurotoxic parameters and histopathological evaluation of the central and peripheral nervous system confirmed no neurotoxic potential for glyphosate.

In conclusion, there was no evidence of specific neurotoxicity up to the highest dose tested (2000 mg/kg bw). Therefore, the no observed effect level (NOAEL) for neurotoxicity, following single oral administration of glyphosate acid, was 2000 mg/kg bw. The NOAEL for systemic toxicity was concluded to be 1000 mg/kg bw/day.

MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24 ([redacted] reference number: Y04707/034)
Purity:	95.6 % w/w
Stability of test compound:	The test substance was shown to be stable for the period of use.
2. Vehicle and/ or positive control:	Deionised water

3. Test animals:

Species:	Rats
Strain:	Alpk:AP$_f$SD (Wistar-derived)
Source:	[redacted]
Age:	At least 28 days
Sex:	Males and females
Weight at dosing:	♂ 171.4 – 175.0 g; ♀ 144.6 – 148.7 g
Acclimation period:	Approx. 2 weeks
Diet/Food:	CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK), *ad libitum*, except 24 h prior dosing

Water:	Tap water, *ad libitum*
Housing:	In groups of five, separated by sex, in multiple rats racks.
Environmental conditions:	Temperature: 19 – 23°C Humidity: 40 – 70 % Air changes: 25 – 30/hour 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: Not reported. The study was conducted during May and June 1995.

Animal assignment and treatment
In an acute neurotoxicity study groups of ten male and ten female Alpk:APfSD (Wistar derived) rats were administered with a single oral dose of 0, 500, 1000 or 2000 mg/kg bw glyphosate acid by gavage.

Dosing Formulation Analysis
Verification of the achieved concentrations was done with samples of each preparation. Homogeneity was determined with samples from the low to high dose levels. The chemical stability of glyphosate acid in water was also determined for all dose formulations over a period of 10 days.

Clinical observations
Clinical observations were made prior to administration and daily thereafter. Any abnormalities together with the observation of no abnormality detected were recorded**.**

Body weight
The body weight of each rat was recorded on Days -7 and -1, immediately before dosing (Day 1), approximately 6 hours after dosing (Day 1) and on Days 8 and 15.

Food consumption
Food consumption for each cage of rats was recorded throughout the study and calculated on a weekly basis**.**

Functional Observational Battery
Prior to the start of treatment (Week -1) and on Day 1 (at approximately 6 hours after dosing), 8 and 15, all animals were observed for signs of functional/behavioural toxicity with quantitative assessment of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hindlimb grip strength). Detailed clinical assessments and functional performance tests were performed together with an assessment of sensory reactivity to different stimuli. Those included but were not limited to the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour; reactivity to stimuli; changes in level of arousal; sensorimotor responses; alterations in respiration.
Locomotor activity was also assessed at these time points. Each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimise disturbances.

Sacrifice and pathology
At scheduled termination, 5 rats/sex/group designated for neuropathology were sacrificed. Brain weight, brain length and brain width were determined. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve.
Submitted tissues were processed as follows: brain (seven levels including the cerebral cortex, the hippocampus, the cerebellum, the pons and medulla), dorsal root ganglia and spinal roots from cervical and lumbar regions of the cord after decalcification, and gastrocnemius muscle from rats receiving either 0 (control) or 2000 mg/kg bw glyphosate acid were routinely processed, paraffin wax embedded and 5µm thick sections were cut and then stained with haematoxylin and eosin. Sections of brain and cord were in the transverse plane.

The Gasserian ganglion, sciatic nerve, spinal cord (cervical and lumbar portions), sural and tibial nerve from

control and high dose group rats were processed and then embedded in Araldite. Semi-thin sections were cut and then stained with toluidine blue. For bilateral tissues only the left was processed. All tissues were sectioned in the transverse plane except the sciatic nerve which was sectioned in both the transverse and the longitudinal plane.

Neuropathological examination was performed on control and highest dose group animals only. All sections were examined by light microscopy.

Statistics
Analyses of variance and covariance were carried out using the GLM procedure in SAS (1989). Least-squares means for each group were calculated using LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squared mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

The levels of probability chosen as significant different from control were $p < 0.01$** and $p < 0.05$* (Student's t-test, two-sided).

II. RESULTS

A. DOSING FORMULATION ANALYSIS

The achieved concentrations of glyphosate acid in water were within 3 % of the nominal levels. The homogeneity was considered acceptable, with a deviation from the overall mean values of approximately ± 8 %. The chemical stability was considered satisfactory.

B. MORTALITY AND CLINICAL OBSERVATIONS

Two females were found dead. The first female received glyphosate at 2000 mg/kg bw/day and was found dead on day 2 of the study which showed treatment-related clinical signs including subdued behaviour, decreased activity, hunched posture, sides pinched in, tip-toe gait and hypothermia on the day of treatment. The death of this animal was attributed to glyphosate exposure. The second female received 500 mg/kg bw/day and showed no clinical signs. As no deaths were observed at the intermediate dose level of 1000 mg/kg bw, the death of this animal was considered not to be treatment related.

At 2000 mg/kg bw/day three surviving females showed clinical signs including subdued behaviour, decreased activity, hunched posture and/or hypothermia on the day of treatment. Full recovery was apparent by day 2.

Distension of the abdomen was recorded for several males from all treated groups on the day of administration. However, in the absence of any dose relationship, this was not considered to be treatment-related.

C. BODY WEIGHT

No treatment-related effects were observed.

D. FOOD CONSUMPTION

During Week 1, mean food consumption was lower in animals receiving 2000 mg/kg bw glyphosate acid compared to controls, although the difference did attain statistical significance only in females (see **Table B.6.7.1.1-1**). There was no evidence of treatment-related effects in animals receiving 500 or 1000 mg/kg bw.

Table B.6.7.1.1-1: Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of food consumption (g/rat/day) during Week 1

Dose level of glyphosate (mg/kg bw)			
0 (control)	**500**	**1000**	**2000**
Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**

Males			
29.9 ± 0.7	29.0 ± 0.1	30.1 ± 0.4	28.4 ± 0.2
Females			
22.4 ± 1.0	22.2 ± 0.2	22.8 ± 0.3	20.6* ± 0.3

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

E. FUNCTIONAL OBSERVATIONAL BATTERY

Examinations of the functional observational battery did not identify any conclusive treatment- and dose-related effects.

Group mean landing foot splay for males receiving 2000 mg/kg bw was statistically significantly lower than that of concurrent controls at approximately 6 hours after dosing on day 1. However, as pre-experimental values for these animals were low, in comparison with control pre-experimental values, the apparent reduction in landing foot splay for high dose group animals on day 1 was considered not to be attributable to administration of glyphosate acid. Group mean landing foot splay for females receiving 500 mg/kg bw was slightly higher than that of concurrent controls on day 15. However, in isolation, this finding was considered to be unrelated to administration of glyphosate acid.

Table B.6.7.1.1-2 Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of landing foot splay (mm)

Dose level of glyphosate (mg/kg bw)				
	0 (control) **Mean ± SD**	**500** **Mean ± SD**	**1000** **Mean ± SD**	**2000** **Mean ± SD**
Males				
Day -7	50.1 ± 7.3	47.7 ± 7.1	51.6 ±6.2	38.9 ± 5.6
Day 1	54.9 ± 7.6	50.0 ± 7.8	56.8 ± 7.8	**45.6* ± 6.8**
Day8	58.9 ± 8.1	57.3 ± 10.6	58.5 ± 12.1	54.9 ± 3.6
Day 15	62.3 ± 7.6	64.6 ± 6.9	67.9 ± 13.2	59.3 ± 7.3
Females				
Day -7	42.4 ± 7.1	46.6 ± 9.2	38.7 ± 5.1	48.6 ± 8.6
Day 1	47.8 ± 8.1	48.4 ± 7.3	47.5 ± 7.8	51.7 ± 8.4
Day8	51.2 ± 8.0	58.1 ± 9.9	54.7 ± 12.0	56.9 ± 8.9
Day 15	54.1 ± 9.1	**64.0* ± 9.8**	58.6 ± 10.3	56.8 ± 6.7

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

Group mean time to tail flick for males receiving 500 mg/kg bw was slightly higher than that of concurrent controls on day 15. However, considering the lack of dose response this finding was considered to be unrelated to administration of glyphosate acid.

Table B.6.7.1.1-3 Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of time to tail flick (s) in male rats

Dose level of glyphosate (mg/kg bw)				
	0 (control) **Mean ± SD**	**500** **Mean ± SD**	**1000** **Mean ± SD**	**2000** **Mean ± SD**
Day -7	8.1 ± 2.9	7.5 ± 2.5	6.0 ± 2.2	7.5 ± 3.7
Day 1	6.3 ± 1.9	6.5 ± 2.6	5.2 ± 2.4	7.5 ± 2.1
Day8	5.3 ± 2.9	5.8 ± 1.9	6.0 ± 2.2	6.1 ± 1.7
Day 15	7.6 ± 3.1	**10.9* ± 4.3**	6.7 ± 1.9	6.6 ± 2.2

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

Group mean forelimb grip strength for males receiving 500 mg/kg bw was slightly higher than that of concurrent

controls on day 15. However, in the absence of a dose-response relationship this finding was considered to be unrelated to administration of glyphosate acid.

Table B.6.7.1.1-4 Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of forelimb grip strength (g) in male rats

Dose level of glyphosate (mg/kg bw)				
	0 (control) **Mean ± SD**	**500** **Mean ± SD**	**1000** **Mean ± SD**	**2000** **Mean ± SD**
Day -7	520 ± 69	538 ± 68	520 ± 89	488 ± 66
Day 1	733 ± 139	705 ± 117	723 ± 99	728 ± 122
Day8	775± 153	798 ±161	850 ± 135	775 ± 129
Day 15	858 ± 175	**1015* ±193**	915 ± 147	823 ± 93

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

Mean overall motor activity values were slightly lower than those of concurrent controls for males and females which received 2000 mg/kg bw on day 1. However, these differences did not attain statistical significance and were considered too small to be of toxicological importance. Mean overall motor activity values were also slightly lower than those of concurrent controls for males which received 2000 mg/kg bw on day 15. However, in the absence of any treatment-related effects for these animals on day 8, this was considered to be incidental and unrelated to administration of glyphosate acid.

Table B.6.7.1.1-5 Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of motor activity (Overall: minutes 1-50)

Dose level of glyphosate (mg/kg bw)				
	0 (control) **Mean ± SD**	**500** **Mean ± SD**	**1000** **Mean ± SD**	**2000** **Mean ± SD**
Males				
Day -7	208.0 ± 105.2	208.8 ± 92.5	216.7 ± 70.0	238.0 ± 62.1
Day 1	383.1 ± 109.7	356.8 ± 173.5	387.6 ± 177.6	276.5 ± 124.4
Day 8	243.9 ± 83.9	307.9 ± 128.4	268.8 ±139.4	279.1 ± 79.9
Day 15	393.6 ± 155.9	402.1 ± 103.1	323.1 ± 89.6	**290.7* ± 136.1**
Females				
Day -7	291.0 ± 167.9	239.6 ± 127.8	287.4 ± 99.8	288.4 ± 134.0
Day 1	464.1 ± 179.9	396.3 ± 165.0	360.3 ± 115.8	312.3 ± 213.9
Day 8	358.1 ± 122.6	382.0 ± 168.4	341.3 ± 135.5	376.9 ± 73.8
Day 15	323.0 ± 170.8	404.8 ± 159.8	379.9 ± 155.7	394.0 ± 136.1

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

F. PATHOLOGY

Necropsy

No macroscopic findings were detected.

Histopathology

No microscopic findings were considered to be treatment-related.

Assessment and conclusion by applicant:

The study is considered acceptable. There was no evidence of specific neurotoxicity up the highest single dose of 2000 mg/kg bw. Based on the study results the NOEL for acute neurotoxicity, following single oral

administration of glyphosate acid, is 2000 mg/kg bw.

Assessment and conclusion by RMS:

The conclusion made by the applicant is agreed with. The NOAEL for acute neurotoxicity is ≥2000 mg/kg bw/day. The NOAEL for systemic toxicity is 1000 mg/kg bw/day based on the clinical signs and mortality in high dose females.

This conclusion is in line with the previous evaluation.

B.6.7.1.2. 90-day neurotoxicity – study 1

Data point:	CA 5.7.1/002
Report author	[redacted] *et al*.
Report year	2006
Report title	Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat
Report No	2060-0010
Document No	NA
Guidelines followed in study	OECD 424 (1997)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion GRG: Valid, Category 1 Conclusion AGG: The study was conducted in accordance with OECD 424 and is considered to be acceptable.

Full summary

In a subchronic neurotoxicity study, groups of 10 male and 10 female Sprague-Dawley (Crl:CD® (SD) IGS BR) rats were fed diets containing 0, 1000, 5000 or 20000 ppm glyphosate (equal to a mean achieved dosage of 0, 77, 395, or 1499 mg glyphosate/kg bw/day in males and 0, 78, 404, or 1555 mg glyphosate/kg bw/day in females for 90 consecutive days.

Clinical signs, functional observations, bodyweight development and food and water consumption were monitored during the study. Ophthalmoscopic examination was also performed on control group and high dose animals before the start of treatment and during the final week of treatment. Five animals per sex from each dose group were subjected to whole body perfusion with glutaraldehyde: paraformaldehyde, followed by the recording of brain weight. Histopathological examinations of neural tissue were performed on all perfused animals from the high dose and control group animals.

Administration of glyphosate produced no unscheduled deaths and no clinically observable signs of systemic toxicity or neurotoxicity. No treatment-related effects were detected in behavioural assessment, functional performance tests, sensory reactivity, or ophthalmoscopy examinations. Food consumption was decreased in top dose males during the first three weeks of exposure. No other adverse effect on dietary intake or food efficiency or water consumption were detected. In high dose males reduced body weight (gain) >10% was observed. There were no treatment-related changes in brain weight. No macroscopic changes or neuropathological changes within a comprehensive histopathological evaluation were detected which could be attributed to administration of glyphosate.

In conclusion, no evidence of a neurotoxic potential was obtained up to the highest dose of 20000 ppm. Therefore, the no observed adverse effect level (NOAEL) for neurotoxic potential, following dietary

administration of glyphosate for at least 90 days, was 20000 ppm (equal to 1499 and 1555 mg/kg bw/day for males and females, respectively). The systemic NOAEL is concluded to be 5000 ppm (equal to 395 mg/kg bw/day) based on the reduced body weight (gain) and food consumption in high dose males.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	Confirmed for the study period

2. Vehicle and/or positive control: Plain diet

3. Test animals:

Species:	Rats
Strain:	Sprague-Dawley Crl:CD® (SD) IGS BR
Source:	[redacted]
Age:	Approximately 5 - 7 weeks
Sex:	male and female
Weight at dosing:	♂ 115 – 153 g (mean); ♀ 118 – 151 g (mean)
Acclimation period:	Up to 7 days
Diet/Food:	Rodent PMI 5002 (certified) diet (BCM IPS Limited, London, UK) and Rodent Rat and Mouse SQC Ground Diet No. 1 (Special Diets Services Limited, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Four per cage per sex in polypropylene grid-floor cages suspended over trays lined with absorbent paper
Environmental conditions:	Temperature: 19-23°C Humidity: 40-70 % Air changes:at least 15 / hour Photoperiod: 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: August 2005 to February 2006

Animal assignment and treatment

In a subchronic neurotoxicity study, groups of 10 male and 10 female Sprague-Dawley rats were fed diets containing 0, 1000, 5000 or 20000 ppm glyphosate for 90 consecutive days (equivalent to mean achieved dose levels of 0, 77, 395 or 1499 mg/kg bw/day for males, and 0, 78, 404 or 1555 mg/kg bw/day for females).

Dietary admixtures were prepared prior to treatment and then twice during the three month study period (i.e. at approximately monthly intervals). The diet was stored at ambient conditions in labelled, double black plastic bags in labelled, covered plastic bins when not in use.

Prior to the start of treatment, the suitability of the formulation procedure was confirmed by measurement of achieved concentration and homogeneity at concentrations of 20000, 5000 and 500 ppm. Samples were taken of each dietary admixture and were analysed for homogeneity, stability and concentration. The concentration of

glyphosate technical in the dietary admixtures was determined by high performance liquid chromatography (HPLC) using an external standard technique.

Mortality/Morbidity
All animals were observed twice daily, early and late during the working day, for morbidity and mortality.

Clinical observations
All animals were examined for overt signs of toxicity, ill-health or behavioural change once daily. All observations were recorded.

Functional observational battery (FOB)
Prior to the start of treatment and during Weeks 3, 5, 9 and 13, all animals were observed for signs of functional/behavioural toxicity. Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. Observations were carried out at a similar time on each occasion wherever possible. Detailed individual clinical observations were performed for each animal using a purpose built arena. The following parameters were observed: gait, posture, tremors, twitches, convulsions, bizarre/abnormal/stereotypic behaviour, salivation, pilo-erection, exophthalmia, lachrymation, hyper/hypothermia, skin colour, respiration, palpebral closure, urination, defecation, transfer arousal, tail elevation. Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. The examinations included quantitative assessments of muscle weakness (fore- and hind limb grip strength).

Locomotor activity
Locomotor activity was monitored by an automated activity recording apparatus. All animals were tested at weeks 3, 5, 9 and 13 (originally intended for week 2, 4, 8 and 12 in the protocol but this was not possible due to scheduling with other studies). The evaluation period was thirty minutes for each animal (with the exceptions of some females during Week 3, which were assessed for twenty-five minutes). The percentage of time each animal was active and mobile was recorded for the overall period and also during the final 20 % of the period (considered to be the asymptotic period).

Body weight
Individual bodyweights were recorded on Day 1 (prior the start of treatment) and at weekly intervals thereafter. Bodyweights were also recorded prior to terminal kill.

Food consumption and compound intake
Food consumption was recorded for each cage group at weekly intervals throughout the study. Food utilisation was calculated retrospectively.

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

Ophthalmoscopic examination
The eyes of all control and high dose animals were examined pre-treatment and before termination of treatment (during Week 13). Examinations included observation of the anterior structures of the eye, pupillary and corneal blink reflex and, following pupil dilation with a mydriatic, detailed examination of the internal structure of the eye using a direct ophthalmoscope.

Sacrifice and pathology
On completion of the dosing period, all animals were killed by intravenous overdose of sodium pentobarbitone. Five males and five females from each dose group were then perfused with glutaraldehyde: paraformaldehyde.

Following perfusion, all animals (both perfused and non-perfused) were subjected to a gross external and internal necropsy and any macroscopic abnormalities were recorded.

The brain from all perfused animals was weighed prior to immersion in buffered 10 % formalin.

Samples of the following tissues were removed from all perfused animals and were immersed in buffered 10 % formalin for histopathology investigations:

Brain: olfactory bulb, forebrain, centre of cerebrum (including hippocampus), midbrain, cerebellum, pons and medulla oblongata
Dorsal root ganglia: (cervical and lumbar sections)
Dorsal and ventral root fibres: (longitudinal cervical and lumbar sections)
Eyes: (longitudinal sections)
Optic nerve: (longitudinal sections)
Sciatic nerve: proximal (longitudinal and transverse sections)
Tibial nerve: proximal (at the knee) and calf muscle branches - (longitudinal and transverse sections)
Skeletal (calf) muscle: (transverse sections)
Spinal cord: (longitudinal and transverse cervical and lumbar sections)

All tissues from the perfused animals from the high dose and control groups were processed to paraffin wax, sectioned, at a nominal thickness of 5 µm and stained with haematoxylin and eosin for subsequent microscopic examination.

Statistics

Data were processed to give group mean values and standard deviations where appropriate. All data were summarised in tabular form. Where appropriate, quantitative data were analysed by the Provantis™ Tables and Statistics Module. For each variable, the most suitable transformation of the data was found, the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANCOVA and Bartlett's test. The transformed data were analysed to find the lowest treatment level that showed a significant effect, using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response was found, but the data showed non-homogeneity of means, the data were analysed by a stepwise Dunnett (parametric) or Steel (non-parametric) test to determine significant differences from the control group. Finally, if required, pair-wise tests were performed using the Student t-test (parametric) or the Mann-Whitney U test (non-parametric).

Probability values (p) are presented as follows: $p < 0.01$ (**); $p < 0.05$ (*); $p \geq 0.05$ (not significant).

II. RESULTS

A. DOSING FORMULATION ANALYSIS

The initial dietary admixtures were within 3 % of the nominal concentration and showed the test material to be evenly dispersed with the diet matrix. Stability of the test material in the diet matrix at these concentrations, under the conditions of use during the study, was also confirmed as part of these initial investigations. Achieved concentrations following storage for six weeks were within 6% of initial concentrations.

Achieved concentrations of dietary admixtures used on the study were measured on three separate occasions and were between 80-102% of nominal concentration. Homogeneity was also confirmed on these occasions. These results confirmed the continuing accuracy of the formulation procedure.

These results indicate that the mean prepared dietary admixture concentrations were within acceptable limits for the purpose of the study.

B. MORTALITY

There were no unscheduled deaths during the treatment period.

C. CLINICAL OBSERVATIONS

There were no neurotoxicologically significant clinical observations detected during the study or at terminal kill.

Incidents of generalised fur loss, staining of the fur and scab formation detected throughout the control and treatment groups, were considered unrelated to treatment. One female treated with 1000 ppm glyphosate displayed a kink in the tail from Day 79 onwards. This was an isolated finding and considered unrelated to treatment.

D. FUNCTIONAL OBSERVATIONS

Functional observational battery (FOB)

No treatment-related effects were detected in the parameters investigated.

All inter and intra group differences in urination, defecation and transfer arousal scores were considered to be a result of normal variation for rats of the strain and age used and were of no toxicological importance.

Sensory Reactivity Assessment

There were no treatment-related changes in the sensory reactivity parameters investigated.

All inter and intra group differences in sensory reactivity scores were considered to be a result of normal variation for rats of the strain and age used and were of no neurotoxicological importance.

Grip Strength Measurements

There were no statistically significant differences detected for mean forelimb and mean hindlimb grip strength test values for treated animals of either sex, in comparison to controls during the study period.

Motor activity

Motor activity assessments did not reveal any obvious signs of neurotoxicity.

Overall activity for females was increased at all treatment levels on Day 57 and Day 85 when compared to control values ($p < 0.01$). No such effects were evident at the previous time-points for females (see Table below), and in the absence of supporting data to suggest these increases were attributed to treatment, these findings were most probably due to low control values and unrelated to treatment.

Statistically significant increases were also evident for the last 20 % activity for females treated with 1000 and 5000 ppm on Day 57, but this was not apparent at 20000 ppm. No such effects were evident on Day 85 and in the absence of a convincing dose-related response, these findings were considered to have arisen incidentally and were of no toxicological importance.

Effects for males were confined to statistically significant increases in overall activity evident for males treated with 1000 ppm on Day 15 and Day 29 ($p < 0.05$) and males treated with 5000 ppm showed a statistically significant reduction during Day 85 ($p < 0.01$). No effects were evident for males treated with 20000 ppm throughout the treatment periods, and in the absence of a dose-related response, these intergroup differences were considered to be of no toxicological significance.

Table B.6.7.1.2-1: Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat ([redacted], 2006): Motor activity findings (mean ± SD [s])

		Dietary concentration (ppm)							
		Males				Females			
Day	**Assessment**	**0**	**1000**	**5000**	**20000**	**0**	**1000**	**5000**	**20000**
-1	Overall activity	566 2±174.0	570.2±115.8	542.8±126.7	609.3±207.5	484.8±268.7	492.2±189.7	567.0±128.2	675.0±233.8
	Overall mobile	2.40±2.88	1.20±0.92	2.80±1.4	2 30±1.83	1.70±1.77	1.60±1.43	1.30±1.49	1.20±0.79
	Last 20 % activity	14.60±15.63	12.20±12 20	16.30±15.81	14.40±16.05	25.00±39.69	10.30±10.49	7.90±5.32	72.90±83.98
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.10±0.32
15	Overall activity	401 2±119.4	**632.0*±106.93**	543.6±136.6	465.8±243.2	479.0±167.7	444.5±162.3	450.0±239.2	515.2±249.8
	Overall mobile	0.44±0.73	0.78±1.64	0.30±0.48	2.60±3.17	3.20±1.87	2.30±2.21	3.50±4.53	5.60±7.62
	Last 20 % activity	23.8±44.0	9.11±8.31	7.6±10.1	13.20±30.56	55.30±62 58	24.30±35.46	27.10±42.84	82.30±84.54
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.20±0.42	0.10±0.32	0.10±0.32	1.20±1.87
29	Overall activity	431.8±105.3	**594.6*±179.3**	448.5±104.9	423 9±123.8	598.2±178.7	768.7±180.8	648.2±196.0	570.3±197.5
	Overall mobile	0.30±0.67	0.30±0.67	0.10±0.32	0.70±1.06	2.60±2.67	2.60±3.37	3.80±3.68	3.10±3.87
	Last 20 % activity	8.50±7.04	**43.60*±51.88**	8.60±7.76	11.00±8.45	60.20±53 30	7090±63.84	43.60±51.00	75.20±67.80
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.30±0.67	0.20±0.42	0.20±0.63	0.30±0.48
57	Overall activity	496.8±131.5	640.4±195.2	433.2±159.1	524.7±98.2	487.9±170.8	**767.6**±102.2**	**794 9**±84.4**	**528.5**±234.5**
	Overall mobile	0.20±0.42	0.90±1.91	0.30±0.67	0.60±1.07	3.10±4.58	2.30±3.59	4.80±3.05	2.20±2.30
	Last 20 % activity	100 1±293.7	36.50±36 30	24.20±35.99	9 10±10.28	42.90±49.87	**97.80*±48.88**	**104 8*±41.58**	71.40±66.67
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.30±0.48	0.80±1.23	0.10±0.32
85	Overall activity	650 9±119.2	508.2±270.0	**344.4**±174.5**	645 2±98.81	363.8±81.61	**655.2**±156.8**	**637.4**±195.3**	**482.2**±161.7**
	Overall mobile	0.00±0.00	0.30±0.95	0.10±0.32	0 30±0.67	1.50±2.12	0.50±0.97	1.20±1.93	0.90±1.37
	Last 20 % activity	41.60±39.07	40.80±42.48	8.10±9.10	43.70±44.38	12.40±15.44	42.30±50.47	81.50±46.39	40.60±53.87
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.10±0.32	0.00±0.00	0.40±0.97	0.10±0.32

* Statistically significant difference from control group mean at the 5 % level
** Statistically significant difference from control group mean at the 1 % level

E. BODY WEIGHT

A slightly reduction in bodyweight gain was evident during the first two weeks of treatment for females treated with 20000 ppm ($p < 0.05$) and for males treated with 20000 ppm during the first four weeks of treatment ($p < 0.01$ - $p < 0.05$). Although not statistically significant overall bodyweight gain was reduced by 15% in males. Moreover, although no statistically significant effect on body weight occurred by day 91 there was a 12% reduction in body weight in high dose males (463.7 g at 20000 ppm versus 525.5 g in the controls).

Males treated with 5000 ppm also showed occasional lower bodyweight gain up to Week 9. These changes also showed a dose-related response.

No effects were noted for females treated with 5000 ppm or animals of either sex treated with 1000 ppm throughout the treatment period.

Table B.6.7.1.2-2: Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat ([redacted], 2006): Group Mean Weekly Bodyweight Gains (mean ± SD [g])

	Dietary concentration (ppm)							
	Males				**Females**			
Day	**0**	**1000**	**5000**	**20000**	**0**	**1000**	**5000**	**20000**
1-8	63.3±6.8	60.8±10.0	60.7±6.5	**46.1**±4.3**	37.4±8.2	36.9±5.4	41.6±5.7	**30.5*±5.3**
8-15	55.3±6.9	56.8±5.1	51.3±5.3	**41.6**±7.5**	27.5±3.5	25.0±6.0	25.3±8.5	**20.6*±3.7**
15-22	49.5±13.5	49.2±7.3	45.1±9.7	**39.4*±7.5**	21.1±6.5	18.8±4.8	17.9±7.7	18.3±5.8
22-29	43.8±10.1	45.3±7.3	**34.6*±7.2**	**35.4*±6.6**	13.6±6.0	15.9±6.6	13.8±5.9	16.3±5.9
29-36	27.5±5.9	25.3±9.9	25.5±11.8	27.4±4.5	13.5±6.9	11.2±4.7	11.6±6.3	12.1±5.7
36-43	26.5±6.3	33.4±7.5	28.0±5.3	28.1±3.4	13.0±4.3	8.3±4.9	15.2±6.9	9.7±4.9
43-50	20.8±8.3	22.2±6.7	15.4±3.9	20.3±4.6	8.7±4.1	11.9±5.1	10.1±6.8	9.0±6.7
50-57	24.2±5.4	23.4±6.0	18.7±5.4	22.5±6.1	6.4±5.7	7.6±6.2	8.1±7.0	5.9±4.5
57-64	11.1±4.5	13.9±4.2	9.5±4.2	11.3±4.2	7.4±4.5	5.4±5.3	6.2±5.6	9.0±5.2
64-71	22.0±6.7	21.2±5.4	**17.3*±4.0**	**16.1*±3.1**	7.1±3.4	7.0±5.5	6.9±7.6	2.7±4.1
71-78	15.4±5.1	15.2±4.8	16.1±4.8	13.6±4.8	7.9±4.2	5.4±5.4	5.9±5.6	7.0±5.9
78-85	19.4±6.7	17.9±5.3	17.4±4.3	18.1±5.9	3.4±6.5	3.0±5.2	5.8±5.2	1.8±5.0
85-91	9.8±4.9	9.6±5.1	11.7±5.5	9.8±3.8	2.2±8.1	6.8±5.1	6.8±5.1	3.2±7.1
Abs. Gain: 1-91	388.6±57.9	394.2±51.6	351.3±36.3	329.7±37.9	169.2±23.5	175.2±22.7	175.2±22.7	146.1±13.1
% Gain: 1-91	283.8±38.4	287.5±37.5	263.0±27.7	247.2±34.7	128.7±17.8	132.4±17.9	132.4±17.9	111.5±12.5

* Statistically significant difference from control group mean at the 5 % level
** Statistically significant difference from control group mean at the 1 % level

F. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE

Dietary intake was slightly lower than the controls during the first two weeks of treatment for males treated with 20000 ppm. Thereafter, recovery of food intake was apparent, although food consumption for males still tended to be marginally lower than controls throughout the treatment period. For females at this level, there was a suggestion of lower intake during the first two weeks of treatment, however, differences from controls were minimal and food intake thereafter was essentially similar to controls.

Food consumption for both sexes at 5000 and 1000 ppm were considered to have been unaffected by treatment.

Table B.6.7.1.2-3: Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat ([redacted], 2006): Group Mean Weekly Food Consumption (g/animal/day)

	Dietary concentration (ppm)							
	Males				**Females**			
Week	**0**	**1000**	**5000**	**20000**	**0**	**1000**	**5000**	**20000**
1-2	25.4	25.5	24.8	21.5 (-15%)	19.3	19.6	20.3	17.6
2-3	27.9	28.0	26.4	23.1	19.0	18.7	20.0	17.5

				(-17%)				
3-4	25.4	29.0	26.6	26.1	18.6	18.9	19.7	18.1
4-5	25.3	30.0	27.9	25.5	20.0	20.0	20.6	20.4
5-6	30.3	29.4	27.2	25.7	20.4	20.5	21.4	20.2
6-7	29.3	30.9	27.9	26.6	20.7	20.7	21.3	20.4
7-8	29.4	30.7	28.0	27.4	20.8	21.3	21.7	20.2
8-9	29.4	30.8	28.1	26.9	20.6	20.4	21.5	19.9
9-10	29.3	30.5	27.9	25.2	20.4	19.9	20.6	19.8
10-11	29.5	30.4	28.5	26.8	20.4	19.6	19.9	19.4
11-12	32.5	30.8	28.7	27.0	20.4	19.4	20.5	19.7
12-13	26.5	29.8	28.6	27.3	20.0	19.3	20.4	19.0
13-13	29.1	30.0	28.6	27.2	18.9	18.8	20.2	18.4

There was no clear effect on food conversion efficiency at any of the dietary inclusion levels investigated, despite the initial effect observed on bodyweight gain and food consumption at 20000 ppm. This finding suggests that the initial reduced bodyweight gain and food intake at this inclusion level could be attributable to slight unpalatability of the dietary admixture.

The mean doses received for males and females respectively were 77, 395, 1499 and 78, 404, 1555 mg glyphosate /kg bw/day at dose levels of 1000, 5000 and 20000 ppm, respectively

Daily visual inspection of water bottles did not show any overt intergroup differences.

G. OPHTHALMOSCOPY

No treatment-related intergroup differences were detected.

The incidental finding recorded for one control animal (female number 11) at the pre-test observation (diffuse opacity of the right eye) was consistent with a low incidental normal finding in young rats of the strain employed and had resolved by the end of the treatment period.

H. PATHOLOGY

Brain measurements

A slight increase in brain weight, both absolute and relative to terminal bodyweights, was evident for males treated with 20000 ppm ($p < 0.05$). This was most probably a consequence of the slight reduction in bodyweight gain evident during the first weeks of treatment. In the absence of any histopathological correlates, these differences were considered to be of no toxicological importance.
No effect was evident for females treated with 20000 ppm or for animals of either sex treated with 5000 or 1000 ppm.

Table B.6.7.1.2-4: Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat ([redacted], 2006): Group Mean Brain Weights with Corresponding Relative (% of bodyweight) Brain Weight

		Dietary concentration (ppm)							
		Males				**Females**			
Week		**0**	**1000**	**5000**	**20000**	**0**	**1000**	**5000**	**20000**
Terminal Bodyweight[#]	Mean [g]	535.4	528.6	486.6	480.6	314.8	295.0	302.6	270.4
	SD	77.4	49.9	47.9	26.9	20.1	17.7	23.4	12.9
	N	5	5	5	5	5	5	5	5
Brain (Including Cerebrum. Cerebellum and Pons	Mean [g]	2.087	2.113	2.075	**2.158***	1.939	1.908	1.876	1.940
	SD	0.091	0.082	0.070	0.032	0.023	0.115	0.064	0.055
	N	5	5	5	5	5	5	5	5
	Mean [%]	0.395	0.402	0.429	0.450*	0.619	0.647	0.623	0.719
	SD	0.048	0.038	0.039	0.022	0.049	0.024	0.055	0.048
	N	5	5	5	5	5	5	5	5

[#]: numbers may slightly differ compared to the study report due to rounding.

Necropsy

No macroscopic abnormalities were detected in control or treatment group animals at terminal kill.

Histopathology

Histopathological examination of the selected tissues from animals of either sex treated with 20000 ppm did not show any treatment-related pathological changes.

Study conclusion:
Dietary administration of the test material, glyphosate technical, to rats for a period of ninety consecutive days at dietary concentrations of up to 20000 ppm did not result in any neurotoxic effects. The "No Observed Adverse Effect Level" (NOAEL) for neurotoxicity was, therefore, considered to be 20000 ppm.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered acceptable. No evidence of a neurotoxic potential was observed up to the highest dose of 20000 ppm. The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm, corresponding to 1499 / 1555 mg/kg bw/day for males and females, respectively.

Assessment and conclusion by RMS:

The neurotoxic NOAEL as concluded by the applicant is agreed with.

The systemic NOAEL is concluded to be 5000 ppm (equal to 395 mg/kg bw/day in males and 404 mg/kg bw/day in females) based on the reduced body weight (gain) and food consumption in high dose males.

B.6.7.1.3. 90-day neurotoxicity – study 2

Data point:	CA 5.7.1/003
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Subchronic Neurotoxicity Study In Rats
Report No	[redacted]/P/4867
Document No	NA
Guidelines followed in study	Study was pre-guideline, but satisfies in general the requirements of OECD 424 (1997)
Deviations from current test guideline	The following deviations were noted: - Functional tests were conducted at -1, 5, 9 and 14 instead of prior to exposure, during the first and second week and monthly thereafter
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion GRG: Valid, category 2a Conclusion AGG: Due to the deviation noted from the current test guideline the study is concluded to be acceptable but with restrictions (reliable with restrictions).

Full summary

In a subchronic neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks.

All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed at intervals. Locomotor activity was also monitored at intervals. At the end of the study, 6 rats/sex/group were killed and subjected to a full post mortem examination. Selected nervous system tissues were removed, processed and examined microscopically.

Administration of glyphosate acid produced treatment-related effects on body weight gain and food utilisation in males receiving 20000 ppm, with no associated effects on food consumption. There were no treatment-related effects on bodyweight, food consumption or food utilisation for males receiving 2000 or 8000 ppm, or for females from all treated groups.

There were no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. In addition, there were no treatment-related changes in brain weight, length or width. Comprehensive histopathological evaluation of the peripheral and central nervous system showed no evidence of any changes which could be attributed to administration of glyphosate acid.

In conclusion, no evidence of a neurotoxic potential was obtained up to the highest dose of 20000 ppm. Therefore, the no observed effect level (NOAEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm (equal to 1546.5 and 1630.6 mg/kg bw/day for males and females respectively). The NOAEL for systemic toxicity is concluded to be 8000 ppm (equal to 1546.5 mg/kg bw/day) based on reduced body weight gain in males at 20000 ppm.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:	
Identification:	Glyphosate acid (technical)
Description:	White solid
Lot/Batch #:	P24 ([redacted] reference number: Y04707/034)
Purity:	95.6%
Stability of test compound:	Confirmed for the study period
2. Vehicle and/ or positive control:	Plain diet
3. Test animals:	
Species:	Rats
Strain:	Alpk:APfSD
Source:	[redacted]
Age:	At least 6 weeks
Sex:	male and female
Weight at dosing:	♂ 215.0 – 218.6 g (mean); ♀ 173.5 – 178.8 g (mean)
Acclimation period:	Approximately 2 weeks
Diet/Food:	CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum* (except up to 24 hours prior to dosing)
Water:	Tap water, *ad libitum*
Housing:	Four per cage per sex in stainless steel cages (26.5 x 50.0 x 20.7cm)
Environmental conditions:	Temperature: 19-23°C Humidity: 40-70 % Air changes: 25-30/hour Photoperiod: 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-05-09 to August 1995

Animal assignment and treatment
In a subchronic neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks (equivalent to mean achieved dose levels of 0, 155.5, 617.1 and 1546.5 mg/kg bw/day for males, and 0, 166.3, 672.1 and 1630.6 mg/kg bw/day for females) glyphosate technical.

All diets were based on CT1 diet supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK. The experimental diets were prepared in 30 kg batches by direct addition of the test substance to 30 kg of CT1 diet and mixing thoroughly. The diets were stored at room temperature until required for use.

Samples from all dietary levels (including controls) were taken at intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet, under the conditions of storage used on this study, was determined for 2000 ppm and 20000 ppm diets prepared for use on a concurrent 1 year feeding study in the rat in the same laboratory.

Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded. A detailed physical examination was performed on each rat prior to start of treatment, and at weekly intervals thereafter.

Functional observational battery (FOB)
Prior to the start of treatment and during Weeks -1, 5, 9 and 14, all animals were observed for signs of

functional/behavioural toxicity. The assessment involved observations in the home cage and/or while the rat was moving freely in a standard arena followed by manipulative/in hand tests. Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. The examinations included quantitative assessments of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hind limb grip strength). The clinical observations included, but were not limited to, the following list: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour; reactivity to stimuli; changes in level of arousal; sensorimotor responses; alterations in respiration.

Locomotor activity
Locomotor activity was monitored by an automated activity recording apparatus. All animals were tested at weeks -1, 5, 9 and 14. Each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimise disturbances.

Body weight
Individual body weights were recorded in week -1, immediately prior to treatment, at weekly intervals thereafter, and at necropsy.

Food consumption and compound intake
Food consumption was recorded as required for each cage group throughout the study and calculated on a weekly basis. Food utilisation and compound intake were calculated.

Water consumption
Not reported.

Ophthalmoscopic examination
Not performed. However, ophthalmological data are available from other repeated dose studies.

Sacrifice and pathology
At the scheduled termination, all main study animals not required for neuropathology, were killed by overexposure to rising concentrations of carbon dioxide gas and were discarded without examination.

At termination, the six rats/sex/group designated for neuropathology were deeply anaesthetised with intraperitoneal sodium pentobarbitone and killed by whole body perfusion fixation with modified Karnovsky's solution. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve.

Brain weight, brain length and brain width were determined.

Submitted tissues were processed as follows: brain (seven levels including the cerebral cortex, the hippocampus, the cerebellum, the pons and medulla), dorsal root ganglia and spinal roots from cervical and lumbar regions of the cord after decalcification, and gastrocnemius muscle from rats receiving either control diet or diet containing 20000 ppm glyphosate acid were routinely processed, paraffin wax embedded and 5µm thick sections were cut and then stained with haematoxylin and eosin. Sections of brain and cord were in the transverse plane.

The Gasserian ganglion, sciatic nerve, spinal cord (cervical and lumbar portions), sural and tibial nerve from control and high dose group rats were processed and then embedded in Araldite. Semi-thin sections were cut and then stained with toluidine blue. For bilateral tissues only the left was processed. All tissues were sectioned in the transverse plane except the sciatic nerve which was sectioned in both the transverse and the longitudinal plane.

Neuropathological examination was performed on control and highest dose group animals only. All sections were examined by light microscopy.

Statistics

All data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter

using the GLM procedure in SAS (1989)[1]. Least-squares means for each group were calculated using LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squared mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

The levels of probability chosen as significant different from control were $p < 0.01$** and $p < 0.05$* (Student's t-test, two-sided).

II. RESULTS

A. DOSING FORMULATION ANALYSIS

The achieved mean concentrations of glyphosate acid in diet were within 4% of the nominal levels, with individual values being within 15% of nominal. There were considered acceptable. The homogeneity of the low- and high-dose diets was considered acceptable, with a deviation from the overall mean values of ± 4%. The chemical stability was considered satisfactory.

B. MORTALITY

No deaths occurred during the study.

C. CLINICAL OBSERVATIONS

There were no treatment-related clinical signs of toxicity.

D. FUNCTIONAL OBSERVATIONS

Functional observational battery (FOB)

There were no clinical signs that could be attributed to administration of glyphosate acid.

There was an apparent increase in the incidence of miosis in males receiving 20000 ppm with 22 observations in 5 animals at 20000 ppm and 6 observations in 3 animals in controls. In addition, there was an apparent increase in the incidence of decreased pupil response to light (6 animals versus 1 in control). However, as these signs were seen for several of these males pre-experimentally and were also present at a similar incidence in females with no obvious relationship to treatment, this was considered to be incidental and unrelated to administration of glyphosate acid.

Landing Foot Splay Measurements

There was no evidence of any treatment-related effect on landing foot splay.

Time to Tail-Flick

There was no evidence of any treatment-related effect on time to tail-flick.

Grip Strength Measurements

There was no evidence of any treatment-related effect on forelimb or hind limb grip strength.

Motor activity

There was no evidence of any treatment-related effect on locomotor activity.

During week 5, slightly reduced locomotor activity was recorded on occasions for females receiving 20000 ppm. However, in the absence of any treatment-related effects on motor activity for these animals at other time points during the study, this is considered to be incidental and unrelated to administration of glyphosate acid.

[1] SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. Cary, NC: SAS Institute Inc., 1989

Table B.6.7.1.3-1: Glyphosate Acid: Subchronic Neurotoxicity Study In Rats ([redacted], 1996): Selected motor activity findings

		Dietary concentration (ppm)							
		Males				Females			
Week	**Assessment period (min)**	**0**	**2000**	**8000**	**20000**	**0**	**2000**	**8000**	**20000**
5	1-50	388.7	472.1	335.6	384.4	441.2	379.3	457.8	359.3
9	1-50	304.7	**413.4***	298.4	327.3	512.3	488.9	555.1	557.0
14	1-50	299.4	395.1	292.2	372.8	553.0	512.7	569.3	514.7

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

E. BODY WEIGHT

Group mean bodyweight for males receiving 20000 ppm was statistically significantly lower than that of controls throughout the study. At week 14, group mean bodyweight for these animals was 92.8% that of controls, equating to a reduction in bodyweight gain of approximately 12%.

Group mean bodyweight for males receiving 8000 ppm was also marginally lower than that of controls from weeks 6 to 14. However, these differences did not attain statistical significance and were considered too small to be of biological importance.

For males receiving 2000 ppm, and for females at all dose levels, mean bodyweight was essentially similar to that of concurrent controls throughout the study.

Table B.6.7.1.3-2: Glyphosate Acid: Subchronic Neurotoxicity Study In Rats ([redacted], 1996): Intergroup comparison of bodyweights (g)

	Dietary concentration (ppm)							
	Males				Females			
Week	**0**	**2000**	**8000**	**20000**	**0**	**2000**	**8000**	**20000**
1	216.0	217.0	218.6	215.0	173.5	178.8	175.6	175.3
2	263.5	264.7	264.9	**254.6****	192.7	200.6	196.1	194.3
4	338.2	340.7	339.6	**323.7***	214.3	**228.3****	**224.9****	219.2
8	440.7	440.1	429.1	**405.8****	253.6	262.1	260.4	255.4
14	534.7	532.8	526.5	**496.1****	285.1	291.5	287.9	281.0

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

F. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no effects on food consumption. The efficiency of food utilisation for males receiving 20000 ppm was statistically significantly lower than that of concurrent controls during weeks 1 to 8 (see table below). There were no changes in the efficiency of food utilisation for males receiving 2000 or 8000 ppm or for females from all treated groups.

Table B.6.7.1.3-3: Glyphosate Acid: Subchronic Neurotoxicity Study In Rats ([redacted], 1996): Intergroup comparison of food utilisation (g growth/100 g food)

	Dietary concentration (ppm)							
	Males				Females			
Week	**0**	**2000**	**8000**	**20000**	**0**	**2000**	**8000**	**20000**
1-4	18.13	17.16	16.94	**16.28***	9.42	9.73	9.36	9.61
5-8	11.52	10.69	10.35	**9.93***	5.99	5.55	5.39	5.70

1-13	12.00	11.45	11.38	**10.87****	6.08	6.03	6.06	5.96

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

The mean doses received for males and females respectively were 155.5, 617.1, 1546.5 and 166.3, 672.1, 1630.6 mg glyphosate acid/kg/day at dose levels of 2000, 8000 and 20000 ppm, respectively

G. PATHOLOGY

Brain measurements

There was no evidence of any effects on brain weight, length or width.

Necropsy

There were no macroscopic findings that were considered to be attributable to treatment.

Histopathology

There were no microscopic findings in the peripheral or central nervous system that were considered to be attributable to treatment.

Study conclusion

Dietary administration of glyphosate acid to rats for a period of ninety consecutive days at dietary concentrations of up to 20000 ppm produced evidence of toxicity in the form of reduced growth and reductions in food utilisation for males. Comprehensive histopathological evaluation of the nervous system showed no evidence of any changes in the peripheral or central nervous system which could be attributed to administration of glyphosate acid.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered acceptable. No evidence of a neurotoxic potential was obtained up to the highest dose of 20000 ppm. The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm, corresponding to 1546.5 / 1630.6 mg/kg bw/day for males and females, respectively.

Assessment and conclusion by RMS:

The NOAEL for neurotoxicity as proposed by the applicant is agreed with.
The NOAEL for systemic toxicity is concluded to be 8000 ppm (equal to 1546.5 mg/kg bw/day) based on reduced body weight gain in males at 20000 ppm.

This conclusion is in line with the previous evaluation.

B.6.7.2. Delayed polyneuropathy studies

B.6.7.2.1. Acute delayed neurotoxicity

Data point:	CA 5.7.2/001
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute delayed neurotoxicity study in domestic hen
Report No	[redacted]/C/3122

Document No	NA
Guidelines followed in study	No guideline stated in the report but in general compliance with OECD 418 (1995)
Deviations from current test guideline	The following deviations from the current OECD test guideline were noted: - NTE activity was measured in 3 animals from the treatment and vehicle control group instead of 6.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: Based on the observed limitation the study is concluded to be acceptable but with restrictions (reliable with restrictions).

Twenty birds were given a single dose of glyphosate acid at a level of 2000 mg/kg bw and observed for the following 21/22 days. Twelve negative control (vehicle, distilled water) and twelve positive control (tri-ortho-cresyl phosphate, TOCP, 1000 mg/kg bw) were also dosed. All birds were observed at least twice daily for any changes in clinical condition. Mortality, bird health and clinical signs were recorded at each observation. Following treatment, the birds were examined daily for signs of delayed ataxia. Bodyweight was measured at weekly intervals. Forty eight hours after dosing, pre-determined birds (three from each group) were examined for brain acetylcholinesterase and brain and spinal cord neuropathy target esterase (NTE) activities. At the end of the 21-day post-treatment observation period six birds from each group were examined histopathologically.

No clinical signs of delayed locomotor ataxia were observed in any birds treated with glyphosate acid. Five birds in the positive control group developed clinical ataxia.
Brain acetylcholinesterase activity was slightly depressed in birds treated with glyphosate acid and TOCP. This was considered to be of no toxicological significance. Brain and spinal cord neuropathy target esterase (NTE) activities in birds treated with glyphosate acid were similar to negative controls. In the positive control group NTE activity was significantly reduced.
Over the 21-day post-treatment period body weight gains were observed in the negative control and in the birds dosed with glyphosate acid. Most birds dosed with TOCP lost weight over the same period.
At necropsy, no changes attributable to treatment with glyphosate acid were detected. Histopathological examination revealed no evidence of acute delayed neurotoxicity or any other treatment-related changes in glyphosate acid-treated hens.

In conclusion, oral administration of a single dose of glyphosate acid at dose level of 2000 mg/kg bw did not produce any clinical signs of delayed neurotoxicity when assessed in terms of clinical ataxia. There was no histological evidence of acute delayed neurotoxicity, and there were no significant reductions in neuropathy target esterase levels in the brain and spinal cord. Therefore, the NOAEL for acute delayed neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	White powder
Lot/Batch #:	P24 (Y04707/034/023)
Purity:	95.6% w/w
Stability of test compound:	The test substance was shown to be stable for the period of use.

2. Vehicle and positive control: Vehicle control: Distilled water
Positive control: tri-ortho-cresylphosphate (TOCP, purity: 99.0 %, vehicle: corn oil)

3. Test animals:

Species:	Domestic chicken (Gallus gallus domesticus) female
Strain:	Lohmann Brown (a hybrid brown laying strain)
Source:	[redacted]
Age:	Approximately 12 months
Sex:	Females
Weight at dosing:	1927 – 2215 g
Acclimation period:	Approx. 2 weeks
Diet/Food:	HRC layer ration in pellet form (Parker Bros. Ltd., Lark Mills, Suffolk, UK, *ad libitum* (except overnight starvation prior dosing)
Water:	Tap water, *ad libitum*
Housing:	Floor pens (galvanised steel, concrete floor) measuring 1.8 x 1.4 m. Number of hens per pen not specified.
Environmental conditions:	Temperature: 15 – 17°C Humidity: 79% Air changes: not given in the report but ventilation considered "adequate" by study author 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1996-01-09 to 1996-02-14.

Animal assignment and treatment

Twenty hens were administered glyphosate acid as a single dose of 2000 mg/kg bw by oral gavage. Twelve birds were employed as positive controls and received a single dose of 1000 mg TOCP/kg bw. The negative control group consisted also of 12 hens and received once distilled water also by gavage. The same volume of 10 mL/kg bw was applied to all hens. Treatment was followed by an observation period of 21 or 22 days.

Dosing Formulation Analysis
Verification of the achieved concentrations was done with samples of each preparation. Homogeneity and the chemical stability of glyphosate acid in water was also determined over a period of 2 hours.

Clinical observations
A check for mortality, clinical signs of toxicity, ill health and behavioural changes was made twice daily on all birds.

Body weight
Individual body weights were recorded weekly.

Food consumption
Food consumption was not recorded

Ataxia assessment
Following treatment, hens were examined daily for signs of (delayed) ataxia.

Sacrifice and pathology
Three pre-determined chicken from each group were sacrificed 48 hours after dosing to determine brain cholinesterase, brain neuropathy target esterase and lumbar spinal cord neuropathy target esterase (NTE) activities.

At the scheduled termination, 6 hens from each group were selected for necropsy and histopathological examinations. Whereas in the negative control and glyphosate-treated groups the first six birds in numerical order (because of the absence of clinical signs) were employed, care was taken in the TOCP-treated group to include all animals that had shown clinical ataxia. The remaining hens from all three groups were killed and discarded.

At termination, after perfusion through the heart with fixative, head and spinal column (with brain and spinal cord exposed but left in place) and dissected sciatic nerves (including tibial branches) from the six hens/group designated for neuropathology were taken and stored. The following tissues were used to take samples for histological examination: brain (forebrain, mid and hindbrain), spinal cord (upper and lower cervical, mid-thoracic and lumbo-sacral parts), sciatic nerve (proximal and distal, above knee), tibial nerve. One transverse and two longitudinal sections were performed at each level.

Statistics

No statistical analysis was necessary since the results were quite clear and number of animals limited.

II. RESULTS

A. DOSING FORMULATION ANALYSIS

The achieved concentrations of glyphosate acid in water were within 4 % of the nominal levels. The results confirm that the formulations were homogenous and stable during ambient temperature storage for 2 hours, a period representing the maximum time from preparation to completion of dosing.

B. MORTALITY AND CLINICAL OBSERVATIONS

There were two unscheduled deaths during the study. In the test group receiving glyphosate acid, one bird was found dead on day 10 after dosing. This hen had not exhibited any signs of toxicity prior to death. The cause of death was apparently not elucidated but in the absence of clinical signs and isolated occurrence it was concluded that this mortality was unrelated to treatment. In the positive (TOCP) control group, one bird had been severely pecked by other hens and was sacrificed on humane grounds, during the acclimation period.

In the glyphosate-treated and negative control groups, there were no uncommon clinical signs observed. In the positive control group one more hen was pecked and had to be treated by applying Stockholm tar to the wounds.

C. BODY WEIGHT

Group mean body weight increased in the glyphosate-treated and negative control groups but weight loss was observed in the positive controls receiving TOCP.

D. FOOD CONSUMPTION

Food consumption was not recorded.

E. ATAXIA ASSESSMENT

Ataxia was confined to the positive control group receiving 1000 mg TOCP/kg bw. Five of the hens were affected. Signs occurred for the first time between post-observation days 11 and 21. The severity of ataxia ranged from 1 – 5 (No ataxia to continuous staggering gait) in a scale of 0-8.

F. PATHOLOGY

Clinical chemistry

In line with ataxia observations, NTE levels in brain and spinal cord were clearly reduced in the positive control group (by 84 % for brain and by 78 % for spinal cord as compared to negative control group) but no effect was seen in the group receiving glyphosate.
A very low reduction of brain cholinesterase (mean 6% less than in negative control) was seen in the hens that had that received glyphosate which is below the limit of adversity. In the positive control group, the mean decrease in brain cholinesterase activity was 19%. No statistical analysis was possible in the study due to the low number (3) of birds investigated. Taking into account the very low difference to negative control birds and the fact that glyphosate is known not to inhibit cholinesterases, a treatment-related effect is unlikely.

Table B.6.7.2.2-1: Glyphosate acid: Acute delayed neurotoxicity study in domestic hen ([redacted], 1996):

Clinical chemistry – group mean values (n=3 per group)

	Group/treatment		
	Negative control **Mean ± SD**	**Glyphosate acid 2000 mg/kg bw** **Mean ± SD**	**Positive control 1000 mg/kg bw TOCP** **Mean ± SD**
Brain AChE [µmol/g/min]	12.75	11.95 (-6 %)	10.37 (-19 %)
Brain NTE [nmol/g/min]	2232	2436 (+9 %)	**360** (-84 %)**
Spinal cord NTE [nmol/g/min]	544	547 (+1 %)	**118** (-78 %)**

Number in parentheses refer to percentage reduction in relation to negative control
** p<0.01

Necropsy

There were no macroscopic findings that were attributable to treatment.

Histopathology

The evaluation of histological findings is complicated by the fact that axonal degeneration in the spinal cord and peripheral nerves were observed in all three groups in nearly all birds suggesting high background incidence. In the TOCP-treated group, the cerebellum was also affected in five out of six animals (as compared to only one bird in the glyphosate group). Furthermore, axonal degeneration in general was more severe in the positive control group.

Assessment and conclusion by applicant:

The study is considered acceptable. Hens receiving glyphosate showed no occurrence of ataxia nor changes in NTE activity. Thus, no delayed neuropathy was observed for glyphosate. Observations of a positive control group receiving TOCP (1000 mg/kg) confirmed the sensitivity of the test. In conclusion, the NOAEL for acute delayed neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg bw.

Assessment and conclusion by RMS:

The NOAEL for acute delayed neurotoxicity as proposed by the applicant is agreed with. The systemic NOAEL is also concluded to be 2000 mg/kg bw, the highest dose tested.

This conclusion is in line with the previous EU evaluation.

B.6.7.2.2. 21-day delayed neurotoxicity

Data point:	CA 5.7.2/002
Report author	[redacted]
Report year	1987
Report title	A 21 Day Oral Neurotoxicity in Domestic Hen of Glyphosate (technical) of [redacted]
Report No	NA
Document No	NA
Guidelines followed in study	No guideline followed
Deviations from current test guideline	Major deviations from currently adopted OECD 419 (only 21 days treated and no post treatment observation performed). It should be noted that at the time this study was run OECD 419 recommended 90 days exposure but no post exposure observation period. In addition, only 3 animals were used per

	dose group instead of 12 (3 per time point for biochemical determination and six for the post treatment period). NTE activity was not measured.
Previous evaluation	Not 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: Invalid, category 3b Conclusion AGG: Due to the major deviations noted from OECD 419 the study is concluded to be unacceptable.

Groups of three birds per dose were orally administered daily doses of 0, 250, 500, or 1000 mg/kg bw/day glyphosate in corn oil for 21 consecutive days. The hens were examined at least once daily for signs of overt toxicity. Body weights and egg weights were recorded daily. Food consumption was recorded on days -7 and -3 before the study and on days 1, 4, 8, 11, 15, 18 and 21 during the treatment period. Blood samples were collected from alar vein and haematological and biochemical parameters were monitored prior to treatment, on the 11th day of the study and at termination. Spinal cord and sciatic nerve were histologically analysed using Holmes silver stain.

All hens survived the treatment period and did not exhibit any signs of neurotoxicity except one high dose hen showing slight ataxia on day 18. All hens of the highest dose group appeared hunched and lethargic from day 5 to 11. Red liquid and matting of feathers in anogenital region were noticed from day 16 to the end of the study. In the other groups, hens did not exhibit any clinical symptoms. An overall reduction in body weight of about 20 % as well as a decrease in food consumption occurred in the highest dose group. A slight reduction in haematological parameters (haemoglobin, packed cell volume and red blood cell count) was also found in this group. Blood chemistry, gross pathology and histology did not provide indications of adverse effects. Egg weight were unaffected in all hens.

In conclusion, oral administration of glyphosate for 21 consecutive days up to 1000 mg/kg bw/day did not produce neurotoxicity. Systemic toxicity was observed in the 1000 mg/kg bw/day group.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate (technical)
Description:	Not provided
Lot/Batch #:	Not provided
Purity:	Not provided
Stability of test compound:	Not provided

2. Vehicle and positive control: Vehicle control: corn oil

3. Test animals:

Species:	Chicken
Strain:	Gallus domesticus
Source:	[redacted]
Age:	8 - 10 months
Sex:	Females
Weight at dosing:	2110 – 2830 g
Acclimation period:	7 days
Diet/Food:	Poultry feed by Maidc LTD, Bombay, *ad libitum*
Water:	water, *ad libitum*

Housing:	Single in wire mesh battery cages		
Environmental conditions:		Temperature:	not provided
		Humidity:	not provided
		Air changes:	not provided
		Light/dark cycle:	not provided

B: STUDY DESIGN AND METHODS

In life dates: Not provided in the study report.

Animal assignment and treatment
Twelve hens were allocated to four dose groups: control (0 mg/kg bw/day), low (250 mg/kg bw/day), mid (500 mg/kg bw/day, and high (1000 mg/kg bw/day). Glyphosate in corn oil was administered by oral intubation into the crop once daily for 21 days.

Dosing Formulation Analysis
No information provided.

Clinical observations
The hens were examined at least once daily for signs of ill health or overt toxicity.

Body weight
Individual body weights were recorded daily from day -7 of the pre-dose period and throughout the treatment period.

Egg weight
The eggs were collected daily and their weights were recorded.

Food consumption
Group food consumption was recorded on days -7 and -3 before the start of the treatment and on days 1, 4, 8, 11, 15, 18, and 21 during the treatment period.

Ataxia assessment
The hens were examined daily and the findings were recorded for each hen. The following score system was used:

No ataxia	0
Doubtful or minor signs	1 (leg weakness)
Positive paralytic signs	2 (lack of leg coordination, loss of balance, tendency to fall back)
Advanced paralytic signs	3 (inability to walk, ataxia, hyperextension, complete prostration and morbidity)
Death	4

Haematology
Blood samples were collected from alar vein and the haematological parameters were examined prior to treatment, on the 11th day of treatment and at termination of the study. The following parameters were examined: haemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), neutrophils (N), lymphocytes (L), eosinophils (E), basophils (B).

Clinical chemistry
Blood samples were collected from alar vein and the biochemical parameters were examined prior to treatment, on the 11th day of treatment and at termination of the study. The following parameters were examined: serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (SAP), total serum protein, blood urea nitrogen (BUN), cholinesterase (in plasma) (ChE).

Sacrifice and pathology
The hens were killed intravenous injection of pentobarbitone sodium and exsanguinated by cutting cervical blood vessels.
The brain was removed intact and was fixed in 10% formalin. The entire spinal cord along with the vertebral column was excised and fixed in 10% formalin containing formic acid. Both sciatic nerves, together with the proximal part of the pereneal and tibial nerves of each leg were removed along with some muscle and fixed in 10% formalin.
The following tissue from all hens were preserved: brain (intact), spinal cord (entire cord in the vertebral

column), sciatic nerves (disted endes, together with the proximal part of the pereneal and tibial nerves with some muscle attached from leg). Transverse and longitudinal sections were made both sciatic nerves and proximal ends of the pereneal and tibial nerves. The spinal cord and sciatic nerve from all the hens were processed to paraffin wax blocks, sections cut at nominal thickness of 10 µm and stained with Holmes silver stain.

Statistics
No information provided.

II. RESULTS

A. DOSING FORMULATION ANALYSIS

Not information provided.

B. MORTALITY AND CLINICAL OBSERVATIONS

All hens survived the treatment period. Hens of the control, low and mid dose groups did not exhibit any clinical symptoms. All hens of the high dose group appeared hunched and lethargic from day 5 to day 11. Red liquid and matting of feathers in anogenital region was seen in all the hens of the high dose group on day 16 onwards.

C. BODY WEIGHT

Overall reduction in body weight of 18% occurred in the hens of the high dose group only. The body weights of the low and mid dose groups were found to be comparable with those of hens of the control group.

Table B.6.7.2.2-1: A 21 Day Oral Neurotoxicity in Domestic Hen of Glyphosate (technical) of [redacted] ([redacted] 1987): Intergroup comparison of body weight (kg)

Dose level of glyphosate (mg/kg bw/day)				
	0 (control) Mean ± SD	**250 Mean ± SD**	**500 Mean ± SD**	**1000 Mean ± SD**
Day 1	2.31 ± 0.14	2.42 ± 0.22	2.47 ± 0.12	2.37 ± 0.09
Day 5	2.34 ± 0.13	2.40 ± 0.25	2.42 ± 0.08	2.36 ± 0.10
Day 10	2.29 ± 0.14	2.43 ± 0.23	2.41 ± 0.10	2.36 ± 0.13
Day 15	2.31 ± 0.13	2.42 ± 0.24	2.38 ± 0.10	2.27 ± 0.11
Day 20	2.29 ± 0.16	2.38 ± 0.22	2.35 ± 0.12	2.02 ± 0.13
Day 22	2.32 ± 0.12	2.41 ± 0.22	2.38 ± 0.14	1.91 ± 0.11

Egg weights: There was no significant difference in the number of egg laid in control, low and mid dose groups. A reduction in the number of eggs laid in the high dose group was observed (11 eggs versus 17 in the control group).

D. FOOD CONSUMPTION

The food consumption was unaffected in control hens and hens of low and mid dose group. Food consumption was progressively reduced in the high dose group from day 8 compared to the control group.

Table B.6.7.2.2-2: A 21 Day Oral Neurotoxicity in Domestic Hen of Glyphosate (technical) of [redacted] ([redacted] 1987):Intergroup comparison of food consumption (g)

Dose level of glyphosate (mg/kg bw/day)				
	0 (control)	**250**	**500**	**1000**
Day -7 to -1	1880	1740	1590	1830
Day 1 to 7	1760	1680	1810	1770
Day 8 to 14	1690	1870	1750	1310
Day 15 to 21	1830	1940	1710	830

E. ATAXIA ASSESSMENT

No hens exhibited any signs of neurotoxicity during the study except one hen of the high dose group which showed slight ataxia on day 18.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Slight reduction in Hb, PCV and RBC was noted in hens of the high dose group at the end of treatment as compared to those in control hens. The toxicological relevance of these findings are difficult to determine due to the lack of statistical analysis and the low number of animals. No changes in haematological parameters were found in the low and mid dose group.

Table B.6.7.2.2-3 A 21 Day Oral Neurotoxicity in Domestic Hen of Glyphosate (technical) of [redacted] ([redacted] 1987): Haematology – group mean values

Dose level of glyphosate (mg/kg bw/day)				
	0 (control) Mean ± SD	**250 Mean ± SD**	**500 Mean ± SD**	**1000 Mean ± SD**
	After 10 days			
Hb [g %]	9.37 ± 0.37	9.87 ± 0.8	9.77 ± 0.63	10.03 ± 0.27
PCV [%]	29.67 ± 0.68	31.67 ± 1.8	31 ± 1.56	31.33 ± 0.09
RBC [x 10^6/mm³]	3.07 ± 0.18	3.3 ± 0.16	3.23 ± 0.09	3.33 ± 0.18
WBC – total [x 10^3/mm³]	10.43 ± 0.44	11.67 ± 0.63	11.37 ± 0.85	11.97 ± 0.92
N [%]	22.67 ± 3.74	32 ± 3.28	29 ± 5.8	32.33 ± 2.41
L [%]	70.33 ± 3.78	58.67 ± 4.42	62.3 ± 4.42	57.3 ± 5.24
M [%]	6.33 ± 1.22	8.67 ± 1.48	8.33 ± 2.07	9 ± 2.56
E [%]	0.67 ± 0.68	0.67 ± 0.34	0.33 ± 0.34	1.33 ± 0.90
	After 21 days			
Hb [g %]	9.43 ± 0.5	9.83 ± 0.74	10.03 ± 0.6	8.6 ± 0.21
PCV [%]	30.67 ± 1.22	32 ± 1.56	32.33 ± 1.89	28.33 ± 0.68
RBC [x 10^6/mm³]	3.17 ± 0.09	3.33 ± 0.17	3.33 ± 0.27	2.87 ± 0.07
WBC – total [x 10^3/mm³]	9.9 ± 0.56	10.46 ± 0.5	10.63 ± 1.3	12.77 ± 0.65
N [%]	31.33 ± 2.65	24.67 ± 4.34	29.67 ± 5.34	26.67 ± 4.75
L [%]	59 ± 4.24	66 ± 2.56	63.67 ± 6.61	65.67 ± 4.75
M [%]	9 ± 1.56	9 ± 3.28	6 ± 1.18	7.33 ± 1.22
E [%]	0.66 ± 0.34	0.33 ± 0.34	0.66 ± 0.68	0.33 ± 0.34

The levels of SGPT, SAP, BUN, total serum protein and blood sugar were comparable in treated hens compared to the control hens.

H. PATHOLOGY

Necropsy

There were no macroscopic findings that were attributable to treatment.

Histopathology

There were no evidence of neurological changes in the spinal cord and peripheral nerves of treated hens and control hens.

Assessment and conclusion by applicant:

To evaluate delayed neurotoxicity this study is considered to be not valid due to major deviations from the current guideline protocol. However, it can be regarded as supportive, that glyphosate does not elicit delayed neurotoxicity in hens up to a dose of 1000 mg/kg bw/day orally for 21 days.

Assessment and conclusion by RMS:

Due to the limitations noted that study is concluded to be unacceptable and therefore no NOAEL has been derived. However, it is noted that glyphosate does not appear to induce delayed neurotoxicity which is in line with the other neurotoxicity studies.

B.6.7.2.3. Delayed neurotoxicity study from the original DAR

In the original DAR an additional delayed neurotoxicity study was reported in which no signs of neurotoxicity were reported, but was already concluded to be unacceptable during the previous evaluation. The study was conducted with a formulation and not with glyphosate itself. The study report was not submitted by the current applicants as they do not have access to the study. For the sake of transparency the original summary from the DAR is copied below:

[redacted](1988): Report on a 21 day oral neurotoxicity study in domestic hen of Glycel 41 SL of [redacted] Report no and dates of experimental work not given. The report was submitted by the notifier Luxan.
A neurotoxicity study as described above ([redacted] 1987) was conducted using Glycel 41 SL technical. Groups of three hens per sex and dose were orally administered daily doses of 0 (control), 400, 800 and 1600 mg glyphosate/kg bw/day in corn oil for 21 days.
All animals survived the study and did not exhibit any signs of neurotoxicity. Clinical signs were confined to the hens of the highest dose group appearing hunched and lethargic from days 6 or 7 onwards. Red liquid and matting of feathers in anogenital region was noticed from day 16 to the end of the study. An overall reduction in body weight of about 23% as well as a decrease in food consumption occurred in the top dose group. A slight reduction in haematological parameters (haemoglobin, packed cell volume and red blood cell count) and a reduction in the number of eggs were also found in this group. Egg weights and blood chemistry were unaffected in all hens. Gross pathology and histology did not provide remarkable findings.

B.6.7.3. Publications on neurotoxicity

B.6.7.3.1. Publications on neurotoxicity – study 1

Data point:	CA 5.7/001
Report author	Martinez, A. *et al.*
Report year	2019
Report title	Effects of glyphosate and aminomethylphosphonic acid on an isogeneic model of the human blood-brain barrier.
Document No	doi.org/10.1016/j.toxlet.2018.12.013 E-ISSN: 1879-3169
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable (Klimisch Score 1).

In this study, the effect of acute exposure to glyphosate (GPH) on the blood-brain barrier in vitro was investigated based on induced pluripotent stem cells (iPSCs). Two chemical analogues: aminomethylphosphonic acid (AMPA) and glycie (GLY) were used as comparators. Concentrations tested ranged from 0.1 µM to 1000 µM..

I. MATERIALS AND METHODS

Chemicals - Glyphosate (EPA 547 1,000 µg/mL solution), aminomethylphosphonic acid (AMPA) and glycine (GLY) were purchased as analytical grade reagents from Sigma-Aldrich, St. Louis, USA. The purity of the test chemicals was not reported.

Cell culture - Induced pluripotent stem cell line IMR90-c4 iPSC (RRID: CVCL_C437) was purchased from WiCell cell repository (WiCell, Madison, WI). iPSC colonies were maintained on hPSC-grade growth factor reduced Matrigel (C-Matrigel, Corning, Corning, MA) in the presence of Essential 8 medium (E8, ThermoFisher, Waltham, MA).

iPSC differentiation - iPSCs were differentiated into brain microvascular endothelial cells (BMEC). iPSCs were seeded as single cells on T-Matrigel at a cell density of 20,000 cells/cm^2 in E8 supplemented with 10 µM Y-27632. 24 hours after seeding, cells were maintained in E8 for 5 days prior to differentiation. Cells were maintained for 6 days in unconditioned medium (UM: DMEM/F12 with 15 mM HEPES, 20% knockout serum replacement, 1% non-essential amino acids, 0.5% Glutamax and 0.1 mM β-mercaptoethanol). After 6 days, cells were incubated for 2 days in the presence of EC+/+ (EC medium supplemented with 1% platelet-poor derived serum, 20 ng/mL human recombinant basic fibroblast growth factor (bFGF) and 10 µM retinoic acid). After such maturation process, cells were dissociated by Accutase® treatment and seeded as single cells on tissue-culture plastic surface (TCPS) coated with a solution of collagen from human placenta and bovine plasma fibronectin at 80 µg/cm^2 and 20 µg/cm^2, respectively. Twenty-four hours after seeding, cells were incubated in presence of EC-/- (EC medium supplemented with 1% platelet poor derived serum (PDS)) 20 ng/ml human recombinant basis fibroblast growth factor and 10 µM retinoic acid. After such maturation process, cells were dissociated by Accutase® (Corning) treatment and seeded as single cells on tissue-culture plastic surface (TCPS) coated with a solution of collagen from human placenta (Sigma-Aldrich) and bovine plasma fibronectin (Sigma-Aldrich) (80 µg/cm2 and 20 µg/cm2 respectively). Barrier phenotype experiments were performed 48 hours after seeding. Differentiation of iPSCs into neurons was done using an adherent 3-step differentiation method. Co-culture experiments were performed by seeding iPSC-derived BMECs at day 8 of differentiation on inserts juxtaposed over 16-days iPSC-derived neurons. BMECs were maintained in EC medium, whereas neurons were maintained in neuron maturation medium (NMM).

Glyphosate, AMPA and glycine treatment - Dilutions of glyphosate, AMPA and glycine were made immediately before the experiments and maintained in cell medium for 24 or 48 hours. In co-culture experiments, the test compounds were added in the apical chamber at a concentration of 100 µM and incubated for 6 hours. iPSC-derived neuron monocultures exposed to similar concentrations served as controls.

Cell metabolic activity - Following treatment, CellTiter Aqueous® MTS reagent ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) was added to each sample following recommendations by the manufacturer. Cells were maintained for 60 minutes at 37 °C followed by a measurement of absorbance at 490 nm using an ELISA plate reader.
Absorbance obtained from the test samples were subtracted from background absorbance and normalized against controls (untreated cells).

iPSC-derived BMECs barrier function - iPSC-derived BMECs were seeded at a seeding density of 10^6 cells/cm^2 on Transwells (polyester, 0.4 µm pore size, Corning) and coated as previously described. Barrier function was assessed 48 hours after seeding of iPSC-derived BMECs monolayers. Barrier tightness was measured by assessing both the transendothelial electrical resistance (TEER) and paracellular diffusion. TEER was measured using an EVOHM STX2 chopstick electrode. For each experiment, three measurements were performed for each insert and the average resistance obtained was used for the determination of the barrier function.

Fluorescein, glyphosate and mannitol permeability assay - To assess changes in paracellular

permeability, sodium fluorescein was added in the apical (top) chamber at a final concentration of 10 µM. 100 µL aliquots were sampled from the basolateral (donor) chamber every 15 minutes for up to 60 minutes. Each aliquot sample was replaced with 100 µL of cell medium. Fluorescein content in the samples was assessed using a fluorimeter ELISA plate reader.
Glyphosate permeability was assessed by incubating cells in the presence of 100 µM glyphosate dissolved in EC-/- in the apical chamber. Sampling in the basolateral chamber occurred as previously described. For the determination of glyphosate the samples taken were alkalinized with 17 µL of borax solution followed by the addition of 17 µL of 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl) solution for the derivatisation of glyphosate. Samples were allowed to incubate in the dark under gentle shaking for 2 hours. The derivatisation process was terminated by adding 137 µL dichloromethane. The sample was homogenized and centrifuged at 2000 rpms for 5 minutes to separate the organic phase and analysis by spectrophotometry at 265 nm. Blank EC-/- medium was used as the blank, whereas glyphosate dissolved in EC-/- at concentrations ranging from 10 nM to 10 µM was used to establish a standard curve.
For the measurement of mannitol permeability, [^{14}C] D-mannitol was added in the apical chamber with sampling in the basolateral chamber as described previously. Radioactivity was assessed by adding 100 µL sample to 5 mL liquid scintillation cocktail and counted using a Beckman-Coulter LS6500 liquid scintillation counter. The permeability across BMECs monolayers was obtained by calculating the clearance slope from both samples and blank inserts and by the calculation of the Pe value.

Immunocytochemistry - Cells were stained on tissue culture polystyrene (TCPS) plates and fixed with 4% paraformaldehyde. Cells were blocked for 1 hour at room temperature in PBS supplemented with 10% normal goat serum (PBS-G) with 0.2 % Triton-X100 and were then incubated overnight in the presence of claudin-5, occludin, GLUT1 or βIII tubulin. Cells were washed with PBS containing 1% bovine serum album, and incubated in the presence of Alexa Fluor®-488 conjugated secondary antibodies for 1 hour at room temperature. Thereafter the cells were counterstained with DAPI and observed on a Leica inverted epifluorescence microscope. Micrograph pictures were acquired using Leica Acquisition Suite X and processed using ImageJ. Semi-quantitative analysis was done by measuring the average fluorescence intensity of each micrograph picture using the built-in measure tool in ImageJ. Average fluorescence values from negative controls were subtracted from the fluorescence values obtained in the test samples.

Flow cytometry - iPSC-derived BMECs at Day 10 of differentiation were treated with 100 µM glyphosate, AMPA or glycine for 24 hours. Cells were harvested by enzymatic dissociation using Accutase® and fixed with 4% paraformaldehyde. Cells were blocked in PBS-G supplemented with 0.2% Triton-X100 dissolved in PBS for 30 minutes, following by an overnight incubation at 4 °C in primary antibody solution (GLUT-1, SPM498 dissolved in PBS-G). Cells were then washed with PBS containing 1% BSA and incubated in the presence of Alexa Fluor® 555-conjugated antibody. As isotype control, cells were exposed to mouse IgG as primary antibody and analysed using a BD FACSVerse®, with a fluorescence photomultiplier tube (PMT) adjusted to IgG isotype control. Fluorescence intensity for each sample was obtained from a count of 10,000 cellular events. Median fluorescence intensity (MFI, geometric mean) was determined for each sample and corrected against IgG isotype.

Glucose and doxorubicin uptake assay - Glucose uptake assays were performed by incubating cells grown on TCPS in presence of cell medium supplemented with [^{14}C] D-glucose. Cells were incubated for 60 minutes at 37 °C. Afterwards the cells were washed with ice-cold PBS and homogenized with PBS + 0.2 % Triton-X100 for 10 minutes. Radioactivity was assessed by adding 100 µL sample to 5 mL liquid scintillation cocktail and counted using a Beckman-Coulter LS6500 liquid scintillation counter. The doxorubicin uptake assay was performed by pre-incubating iPSC-derived BMECs in the presence of glyphosate, AMPA or glycine at 100 µM for 2 hours. Doxorubicin was added to obtain 5 µM as a final concentration and allowed to incubate for 1 hour. Cells were homogenized as previously described and total fluorescence assessed by fluorimetry. Total protein content obtained from cell homogenates was determined using a BCA protein assay.

Statistical analysis - Cells were randomly assigned treatment conditions prior each experiment. Data are represented as mean ± S.D. from three or more independent experiments. One-way analysis of the variance (ANOVA) coupled with Dunnett (or Kruskal-Wallis) tests analysis were performed using Prism 7.0 built-in package (GraphPad Software). A p-value < 0.05 was considered as statistically significant.

II. RESULTS

BMEC cell viability – For the assessment of the effect of glyphosate on the viability of BMECs cell monolayers a range of concentrations was used partially overlapping the levels found in patients reported as asymptomatic, minor and moderate (17, 241, and 428 µM, respectively). Treatment with glyphosate, AMPA or glycine for 24 hours at concentrations ranging from 10 to 1000 µM resulted in no changes in cell metabolic activity. This indicates that glyphosate and AMPA unlikely have toxicity towards the blood-brain barrier.

Fluorescein permeability in BMECs monolayers - Changes in the barrier function in BMECs monolayers were measured using TEER and fluorescein permeability. In addition to the previous concentrations used, 2 concentrations (0.1 and 1 µM) were included to reflect average plasma concentrations reported in occupational exposure. No changes in TEER were noted for any of the concentrations tested of glyphosate, AMPA or glycine. However, a biphasic response was noticed in fluorescein permeability. At 0.1 µM, a slight but not statistically significant decrease in fluorescein permeability was found for glyphosate, AMPA and glycine followed by a statistically significant increase for both glyphosate and AMPA at 1 and 10 µM but not at 100 and 1,000 µM. Higher concentrations resulted in permeability values similar to controls. To confirm the increase in the paracellular profile of glyphosate, changes in paracellular permeability were investigated using [^{14}C]-mannitol, an alternative paracellular flux marker. A modest but statistically significant increase in mannitol permeability was observed at 10 µM glyphosate. No significant increase was noted following AMPA or glycine treatment. In summary, the data suggest that glyphosate and AMPA treatment may increase the barrier permeability in BMECs monolayers.

Tight junction complexes integrity - To better understand the effect of glyphosate and AMPA on the barrier function, changes in tight junction complexes were investigated, in particular changes in claudin-5 and occludin, by immunocytochemistry. No changes in claudin-5 immunolocalisation were observed. However, a dose-dependent decrease in claudin-5 relative expression was noted in all groups as quantified by fluorescence intensity. Glyphosate decreased claudin-5 fluorescence intensity at 100 and 1000 µM, but treatment with AMPA already decreased significantly claudin-5 fluorescence intensity at 10 µM. No changes in occludin localization occurred following treatment although a significant decrease in occludin protein levels was noted in all treatment groups with the exception of 10 µM glyphosate. Unlike claudin-5, this effect appeared to be dose-independent. Taken together, the data suggest that glyphosate may increase paracellular permeability in BMECs monolayers to fluorescein via partial disruption of tight junction complexes integrity.

Diffusion across the BBB - The ability of glyphosate to cross the blood brain barrier (BBB) was investigated following a single exposure at 100 µM in the apical chamber for 2 hours and measurement of the amount of glyphosate present in the basolateral chamber. After 2 hours of diffusion, the amount of glyphosate capable of crossing BMEC monolayers was about 1.67 ± 0.31 % of the applied dose. It was found that the permeability of glyphosate was significantly greater than fluorescein ($18.67 \pm 3.55 \times 10^{-6}$ cm/min versus 10.59×10^{-6} cm/min) or mannitol ($13.10 \pm 2.03 \times 10^{-6}$ cm/min). In conclusion, the data suggests that GPH may cross the BBB via a transcellular mechanism. Also the effect of glyphosate and AMPA on drug efflux transporters was investigated using doxorubicin as a drug efflux substrate. With the exception of AMPA that showed a 2-fold increase over control, exposure to 100 µM glyphosate or glycine for 24 hours showed no differences compared to controls.

Modulation of glucose uptake in BMECs - As previous studies reported changes in glucose levels in certain vertebrates following exposure to glyphosate and AMPA changes in GLUT1 (the main glucose transporter at the BBB) localization and expression in BMECs following treatment by immunocytochemistry were studied. Following exposure at 100 µM glyphosate, but not at 1,000 µM, an apparent increase in GLUT1 immunoreactivity was noted. Similar results were obtained with AMPA although less pronounced. A flow cytometry analysis was performed where changes in mean fluorescence indexes were compared following exposure at 100 µM for 24 hours. Exposure to glyphosate yielded an increase in GLUT1 expression levels compared to control. Although AMPA showed no differences in GLUT1 expression, glycine exposure resulted in a significant decrease compared to control. Taken together, our data suggest that exposure to high levels of GLY or AMPA may impair glucose uptake and metabolism in BMECs monolayers via an alteration in GLUT1 expression and/or activity.

Barrier function of neurons co-cultured with BMECs - As glyphosate showed the ability to cross the BBB and produced changes in GLUT1 expression and glucose uptake in BMECs monolayers, the effects of glyphosate on neurovascular coupling using a BMEC/neurons co-culture model was investigated. First, the ability of such co-cultures to yield barrier function was assessed by measuring differences in TEER between BMECs monocultures and BMECs co-cultured with iPSC-derived neurons. A 3-fold increase in TEER in BMECs co-cultured with neurons compared to BMECs maintained in monocultures was observed. These co-cultures were then exposed to 100 µM glyphosate, AMPA or glycine for 24 hours. TEER measurements indicated that there was no statistically significant difference in barrier tightness when compared to controls. A mild increase in fluorescein permeability was noted with glyphosate when compared to control, but not with AMPA or glycine.

Neurovascular coupling - The effect of glyphosate on neurovascular coupling was investigated by measuring changes in neural cell metabolic activity using MTS following exposure to 100 µM for 6 hours. A significant decrease in cell metabolic activity was observed in co-cultured neurons when compared to monocultures. When co-cultured neurons were exposed to glyphosate and AMPA the metabolic activity was statistically significantly increased when compared to controls. Glyphosate and AMPA produced no statistically significant changes in metabolic activity of mono-cultured neurons. When the effect of glyphosate, AMPA and glycine was investigated in iPSC-derived neuron colonies by immunocytochemistry against βIII-tubulin, no changes were observed both in monocultures and co-cultures, suggesting that changes in cell metabolic activity is unlikely due to cell death. In conclusion, the data suggest that exposure to high amount of glyphosate (100 µM) impair neurovascular coupling.

Neuron progenitor cells (NPC) - The effect of glyphosate on differentiating and differentiated neurons was investigated by exposing cells to a concentration considered representative of the amount crossing the BBB i.e. 0.1–1 µM. First, the effect of an exposure of 24 hours on the cellular metabolic activity of undifferentiated NPCs was investigated using the MTS assay. A significant decrease in cell metabolic activity was seen at concentrations of glyphosate and glycine of 1 µM although immunofluorescence analysis of these NPCs showed no major alterations in the relative cell density and nestin (a cellular marker of neural stem cells/progenitor cells) immunoreactivity. Then, differentiating NPCs were treated continuously for 16 days (by replacing cell medium every 48 hours) in the presence of 0.1 µM glyphosate, AMPA or glycine. The concentration tested is representative of plasma concentrations reported in occupational workers and is 20 times higher than values reported in non-occupational population. No significant changes in cell metabolic activity were observed between the different groups compared to controls. After 16 days of exposure to glyphosate or AMPA no changes in gross morphology of iPSC-derived neuron colonies were evident. In conclusion, chronic exposure to low levels of glyphosate or AMPA failed to show any signs of neurotoxicity.

Neurites density - The exposure of iPSC-derived neurons seeded at low density (50,000 cells/cm^2) to glyphosate or AMPA for 24 hours at concentrations ranging from 1 µM to 1000 µM decreased statistically significantly cell metabolic activity at 10 µM and beyond. AMPA showed similar results, albeit not statistically significant. Treatment with glycine showed only small effects on cell metabolic activity. Changes in cell density and neurites formation by immunocytochemistry were also investigated and, with the exception of glycine, no depletion in neurites was observed with glyphosate and AMPA. Upon quantification of cell nuclei and neurites per surface area a progressive decrease in neuron density was noted for both glyphosate and AMPA, with a significant decrease at 1000 µM. However, no differences in neurite density were noted with glyphosate and AMPA, with the exception of glycine at 100 and 1000 µM. Taken together, the data suggest that low concentrations (< 10 µM) of GPH and AMPA may not have detrimental effects on iPSC-derived neurons.

Discussion

The toxicity of acute glyphosate poisoning on blood-brain barrier integrity was investigated by assessing its activity on the different cell types of the neurovascular unit. Cells were exposed to glyphosate and AMPA at concentrations ranging from 10 to 1000 µM. This is the concentration range of glyphosate plasma values in patients with self-inflicted poisoning (scored from asymptomatic (17 µM) to fatal (8.12 mM) at the time of admission in clinic). An increase in fluorescein permeability was noted for glyphosate and AMPA at 1 µM and 10 µM. A similar outcome was noted for mannitol in cells exposed to 1 µM glyphosate. This suggests a possible detrimental effect of glyphosate and AMPA on blood-brain barrier function. Although no major changes in tight junction complexes localization were observed, a decrease was found in both claudin-5 and occludin protein levels after exposure to glyphosate, AMPA and glycine, suggesting that glyphosate and AMPA may interfere with tight junction complexes integrity. Yet, the interference of such compounds on tight junction proteins remains unclear.

Although not statistically significant, a 50 % increase in paracellular permeability was noted with glycine at 100 and 1000 µM. The data from this study suggest that high levels of glycine may increase the permeability of the BBB and disrupt tight junction complexes. In addition to changes in barrier function, glyphosate permeability in BMEC monolayers was assessed. It is estimated that about 1 % of the applied dose (100 µM) diffused across BMECs monolayers. However, the permeability for glyphosate was significantly higher than that for fluorescein despite its high hydrophilicity (xLogP = -4.63). This indicates that glyphosate crosses the BBB via carrier-mediated diffusion. Amongst the different cell types, neurons displayed the most important changes in metabolic activity following exposure to glyphosate and AMPA. Significant changes in neuronal cell metabolic activity were observed following exposure to glyphosate, AMPA or glycine whereas such changes we not observed in BMECs. Yet, such decrease in cell metabolic activity was unlikely to be considered as neurotoxicity since these effects didn't translate in changes in neuronal cell density and neurites formation. The change in cell metabolic activity observed may be due to changes in glucose metabolism, as changes in glucose uptake in BMECs, as well as some changes in GLUT1 expression levels were noted.

III. CONCLUSION

The authors concluded that data from this study demonstrate the relative safety of glyphosate and AMPA with regard to the blood-brain barrier after acute exposure with minimal effects observed at concentrations significantly higher than baseline exposure levels, occupational and non-occupational alike. The presence of an active uptake and diffusion of glyphosate across the blood-brain barrier suggests the need of extensive brain-centered studies to evaluate the pharmacokinetics and pharmacodynamics of glyphosate on the central nervous system during acute exposure and in individuals exposed to high amounts of such pesticides.

Assessment and conclusion

Assessment and conclusion by applicant:

The effect of glyphosate, AMPA and glycine was investigated on the integrity of the blood-brain

barrier *in vitro* using an induced pluripotent stem cell line differentiated into brain microvascular endothelial cells (BMEC) and neurons. The endpoints investigated were BMEC cell viability, fluorescein permeability in BMEC cell monolayers, tight junction complexes integrity, diffusion across the blood-brain barrier, modulation of glucose uptake in BMECs, barrier function of neurons co-cultured with BMECs, neurovascular coupling, differentiation of neuron progenitor cells and neurites density. The results of this study indicate that glyphosate or AMPA are unlikely to present toxicity towards the blood-brain barrier. Minimal effects on single parameters were observed with glyphosate or AMPA, but were comparable with effects of the amino acid glycine.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterized and no positive controls were used in any of the assays conducted.

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

Publication: Martinez *et al.*, 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of glyphosate and AMPA not reported. Source: Sigma-Aldrich, St. Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also glycine and AMPA tested.
AMPA is the tested substance	Y	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (<1 mM)	Y	Concentration range *in vitro* from 0.1 to 1000 µM for some tests.
Cytotoxicity tests reported	Y	
Biochemical methods described	Y	Some could be better documented.
Analytical method described	Y	The method for the analysis of glyphosate.
Positive and negative controls	N	No positive controls were used.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	For some tests
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterized and no positive controls were used in any of the assays conducted.		

Assessment and conclusion by RMS:

The study is concluded to be reliable (Klimisch Score 1) using the ToxR Tool.

Overall, the study does not indicate an neurotoxic potential for glyphosate which is in line with the guideline studies available. Also for AMPA, the metabolite of glyphosate the study does not indicate a neurotoxic potential.

B.6.7.3.2. Publications on neurotoxicity – study 2

Data point:	CA 5.7/002
Report author	Martínez, M. et al.
Report year	2018
Report title	Neurotransmitter changes in rat brain regions following glyphosate exposure
Document No	doi.org/10.1016/j.envres.2017.10.051 E-ISSN: 1096-0953
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable with restrictions

Full summary of the study according to OECD format

The effects of glyphosate oral exposure on brain region monoamine levels in male Wistar rats were examined. Glyphosate-treated rats (35, 75, 150 and 800 mg/kg bw, 6 days), had no visible injury, i.e., no clinical signs of dysfunction were observed. After the last dose of glyphosate, the levels of serotonin (5-HT), dopamine (DA) and norepinephrine (NE) and its metabolites were determined in the brain regions striatum, hippocampus, prefrontal, cortex, hypothalamus and midbrain, by HPLC. Glyphosate caused statistically significant changes in the 5-HT and its metabolite 5-hydroxy-3-indolacetic acid (5-HIAA), DA and its metabolites 3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and NE and its metabolite 3-metoxy-4-hydroxyphenylethyleneglycol (MHPG) levels in a brain regional- and dose-related manner. Moreover, glyphosate, dose-dependently, evoked a statistically significant increase in 5-HT turnover in striatum and hypothalamus and in DA turnover in prefrontal cortex and hippocampus, and a statistically significant decrease in NE turnover in prefrontal cortex and hypothalamus. The present findings indicate that glyphosate significantly altered central nervous system (CNS) monoaminergic neurotransmitters in a brain regional- and dose-related manner.

I. MATERIALS AND METHODS

Chemicals; Glyphosate [N-(phosphonomethyl) glycine], molecular formula $C_3H_8NO_5P$ CAS RN 107-83-6, purity ≥ 98%, serotonin (5-HT) and its metabolite [5-hydroxy-3-indolacetic acid (5-HIAA)], dopamine (DA) and its metabolites [3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] and norepinephrine (NE) and its metabolite [3-metoxy-4-hydroxyphenylethyleneglycol (MHPG)] were purchased from Sigma-Aldrich, St Louis, MO, 63103 USA. All other chemicals were of the highest quality grade and obtained from commercial sources.

Animals and experimental design; All experiments using live animals were undertaken in accordance with the ethics requirements and authorized (protocol number 086) by the official ethical committee of our university. Male Wistar rats of 60 days old each weighing 200–210 g (Charles River Inc., Margate, Kent, UK) were used. The animals were individually housed in polycarbonate cages with sawdust bedding and maintained in environmentally controlled rooms (22 ± 2°C and 50 ± 10 % relative humidity) with a 12 h light/dark cycle (light from 08.00 to 20.00 h). Food (A04 rodent diet, Scientific Animal Food & Engineering, SAFE, Augy, France) and water were available *ad libitum*. Thirty male rats were assigned randomly to five groups of 6 animals each, a control group and four glyphosate treated groups. Animal treated groups received glyphosate orally at the dose of 35, 75, 150 and 800 mg/kg bw [equivalent to 1/160, 1/75, 1/37 and 1/7 of the acute oral rat LD50 ≈ 5.6 g/kg bw] for 6 consecutive days. The doses were chosen taking into account the LD50 oral value as well as the NOAEL (no observed adverse effect level) described in the literature. The glyphosate treated group rats were deprived of food for 6 h before the oral administration of glyphosate, but were allowed water ad libitum. Glyphosate was dissolved in water and was administered orally by gavage in a maximum volume of 2 mL/rat. Control animals received the vehicle (water) on the same schedules. The animal body weights were measured during the study and food and water consumption of each animal was also assessed. The animals received the treatment at the same time each day, specifically between 10:00 and 11.00 a.m. Three hours after the last dose, the animals were sacrificed by decapitation. The brain was removed quickly and hypothalamus, midbrain, hippocampus, striatum and prefrontal cortex tissues rapidly dissected out at 4 °C. Tissues were rapidly weighed and stored at -80 °C until analysis.

Determination of monoamine levels; The five brain regions analysed in the present study were hypothalamus, midbrain, hippocampus, striatum and prefrontal cortex. Following sample collections, 300–800 μL of 0.4 M $HClO_4$ containing 0.1 % (w/v) $Na_2S_2O_5$ was added to the tissues, and the mixture was homogenized (1 min) by sonication (Labsonic U-Braun). The homogenates were centrifuged (RC5C, Sorvall Instruments) at 12,000g for 20 min at 4 °C and aliquots of supernatants were taken for analysis of 5-HT and its metabolite 5-HIAA, DA and its metabolites DOPAC and HVA and NE, using a high performance liquid chromatography (HPLC) technique with electrochemical detection. Also, aliquots of supernatants were taken for analysis of the norepinephrine metabolite MHPG by HPLC with fluorimetric detection. An acid-catalysed procedure was used to hydrolyse MHPG-sulphate in homogenates of brain region tissues. Volumes of 200–300 μL of the supernatants (in 0.4 M $HClO_4$) were treated for 3 min at 100 °C in a water bath. The samples were then cooled and 30 – 45 μL of 2 M NaOH were added (final pH: ca. 1.5) and aliquots were injected into a reverse phase HPLC system.

For the analysis of catecholamines NE, DA, DOPAC and HVA, the mobile phase consisted of 0.1 M $Na_2HPO_4 \cdot 2H_2O$, 0.1 M citric acid (pH 3.5), 1.6 mM octane sulphonic acid, 0.9 mM EDTA and 10 % (v/v) methanol. For the analysis of the indolalkylamines 5-HT and 5-HIAA, the mobile phase consisted of 0.1 M $Na_2HPO_4 \cdot 2H_2O$, 0.1 M citric acid (pH 3.5) and 10 % (v/v) methanol. Elution was performed at a flow rate of 1 mL/min and the working electrode potential was set at 0.8 V for catecholamines and 0.7 V for indolalkylamines. The HPLC system consisted of a Shimadzu liquid chromatograph, model LC-9A equipped with a 5 μm particle size C18-Nucleosil reversed phase column (4 mm i.d. × 125 mm) proceeded by a C18 pre-column, an electrochemical detector (Shimadzu, model L-ECD-6A), a sample injector (20 μL valve) and an integrator (Shimadzu, model C-R6A Chromatopac). For the analysis of the norepinephrine metabolite (MHPG), the mobile phase consisted of 0.06 M $Na_2HPO_4 \cdot 2H_2O$, 0.03 M citric acid and 6 % (v/v) methanol. Elution was performed at a flow rate of 1.5 mL/min. The HPLC system consisted of a Shimadzu liquid chromatograph, model LC-10AS, a 25 μm particle size Tracer Extrasil ODS reversed phase column (4 mm i.d. × 125 mm), a fluorescence detector (Shimadzu, model RF-551), a sample injector (20 μL valve) and an integrator (Shimadzu, model C-R6A Chromatopac). Excitation and emission wavelengths of the detector were 275 and 315 nm, respectively.

Peak areas from the sample chromatograms were used to quantify the analytes by external standard technique using solutions of catecholamines (NE, DA, DOPAC y HVA), indolalkylamines (5-HT and 5-HIAA) and norepinephrine metabolite (MPHG) reference standards (Sigma Chemical Co., St Louis, MO, USA). For tissue specimens as determined by use of a linear least squares regression procedure, a linear relationship existed in the calibration curve of catecholamines (NE, DA, DOPAC, HVA), indolalkylamines (5-HT, 5-HIAA) and norepinephrine metabolite (MPHG) over the range of 0.002–

100 µg/g, which always yielded a correlation coefficient exceeding 0.9998. Overall mean recovery of catecholamines (NE, DA, DOPAC and HVA), indolalkylamines (5-HT and 5-HIAA) and norepinephrine metabolite (MPHG) from tissues was 100 % for every analyte. Within- and between-day variation was < 4 %. Quantification limit (LOQ) was 2 ng/g for NE, DA, DOPAC, 5-HT and 5-HIAA and 10 ng/g for HVA and MPHG in the different tissue matrices. NE, DA and 5-HT turnover were calculated as ratios of metabolites to neurotransmitter.

Data analysis; Statistical analysis of data was performed using GraphPad Prism 6 for Windows. Results are presented as mean ± S.D. of 6 animals per group. Results significantly different from controls are also presented as percentage change over control. One-way ANOVA was carried out to determine significant dose-dependent effect of glyphosate on 5-HT, DA, NE and metabolite levels and the corresponding turnover values in the brain regions studied, followed by Tukey's post hoc test. Statistical significance was set at $P < 0.05$. ANOVA's F values are presented in the Tables. F distribution was calculated with numerator degrees (DFn) and denominator degrees of freedom (DFd).

II. RESULTS

The glyphosate-treated rats at oral doses of 35, 75, 150 and 800 mg/kg bw/day for 6 days had no visible injury. These doses were selected based on preliminary experiments where the doses and route of administration did not show any adverse effects, abnormal clinical signs as well as changes in body weight, food and water consumption in the animals (see table below).

Table B.6.7.3.2-1: Effect of glyphosate on body weight gain and food and water consumption in male rats

Parameter	Animal groups				
	Control	Glyphosate (35 mg/kg bw, 6 days)	Glyphosate (75 mg/kg bw, 6 days)	Glyphosate (150 mg/kg bw, 6 days)	Glyphosate (800 mg/kg bw, 6 days)
Body weight gain (g)	29.17 ± 2.64	27.67 ± 4.23	29.33 ± 3.08	30.00 ± 4.00	27.33 ± 4.50
Food consumption (g)	96.33 ± 7.45	98.00 ± 7.27	95.66 ± 7.39	93.67 ± 9.24	95.50 ± 9.05
Water consumption (mL)	133.83 ± 20.92	131.50 ± 20.12	129.5 ± 21.50	140.50 ± 24.77	151.00 ± 9.19

Results are presented as means ± SD for six rats.
Results are not significantly different from control group.
Continuous probability distribution (F) for all parameters were lower than 1.140 (DFn = 4, DFd = 25).

All the rat groups exposed to glyphosate by oral route did not show statistically difference on weight of tissues (brain regions) or the ratio weight tissue/body weight (%) compared to control group (data not shown).
Glyphosate at a dose of 35 mg/kg bw did not affect the 5-HT, DA, NE and metabolite levels in the brain regions studied. In this study, 35 mg/kg bw might be identified as the NOAEL based on neurotransmitter changes in CNS.
Glyphosate at doses of 75, 150 and 800 mg/kg bw produced in a dose-dependent manner a significant decrease of 5-HT content respect to control in striatum. Moreover, glyphosate at doses of 150 and 800 mg/kg bw produced a significant decrease of 5-HT content respect to control in hippocampus and prefrontal cortex and only at a dose of 800 mg/kg bw in hypothalamus and midbrain. In addition, the highest dose (800 mg/kg bw) of glyphosate resulted in a significant decrease in the 5-HIAA levels in hippocampus compared to control group. Also, glyphosate at doses of 150 and 800 mg/kg bw significantly increased the turnover (5-HIAA/5-HT) in striatum and hypothalamus compared to control groups (see table below).

Table B.6.7.3.2-2: Effect of glyphosate on 5-HT and 5-HIAA levels and turnover (5-HIAA/5-HT) in brain regions of male rats

Parameter	Animal groups: oral dose of glyphosate during 6 consecutive days	Brain regions				
		Striatum	Hippocampus	Prefrontal cortex	Hypothalamus	Midbrain
5-HT (ng/g)	Control	696.28 ± 14.11	321.60 ± 12.99	710.24 ± 39.86	1698.90 ± 15.31	1551.79 ± 70.09
	35 mg/kg bw	650.05 ± 38.04	319.00 ± 8.17	675.01 ± 49.65	1642.00 ± 74.65	1541.00 ± 62.59
	75 mg/kg bw	557.50 ± 112.42** (−20)[a]	315.16 ± 6.98	603.64 ± 133.99	1575.00 ± 147.39	1515.42 ± 51.99
	150 mg/kg bw	445.57 ± 85.33*** (−36)[a]	293.97 ± 6.23* (−9)[a]	507.20 ± 42.11*** (−29)[a]	1360.75 ± 452.82	1495.03 ± 64.02
	800 mg/kg bw	355.11 ± 16.48*** (−49)[a]	291.06 ± 27.67* (−9)[a]	482.80 ± 69.26*** (−32)[a]	1103.07 ± 111.29*** (−35)[a]	1210.46 ± 134.13*** (−22)[a]
		27.45[b]	5.793[b]	10.51[b]	7.330[b]	18.18[b]
5-HIAA (ng/g)	Control	365.50 ± 75.88	390.31 ± 99.55	299.85 ± 48.05	743.32 ± 78.12	865.34 ± 123.9
	35 mg/kg bw	366.30 ± 55.29	383.04 ± 6.35	297.00 ± 48.98	755.00 ± 33.45	846.00 ± 95.25
	75 mg/kg bw	366.49 ± 68.09	351.54 ± 8.99	295.10 ± 64.32	736.09 ± 55.73	820.32 ± 120.66
	150 mg/kg bw	368.70 ± 31.54	307.93 ± 91.06	270.69 ± 58.58	733.13 ± 116.03	805.00 ± 66.93
	800 mg/kg bw	352.41 ± 60.91	269.81 ± 55.41* (−31)[a]	259.39 ± 37.20	726.33 ± 40.54	708.82 ± 151.74
		0.06677[b]	3.669[b]	0.7277[b]	0.175[b]	1.661[b]
5-HIAA/5-HT	Control	0.53 ± 0.12	1.22 ± 0.30	0.42 ± 0.06	0.45 ± 0.04	0.56 ± 0.09
	35 mg/kg bw	0.56 ± 0.06	1.20 ± 0.04	0.45 ± 0.11	0.47 ± 0.20	0.55 ± 0.08
	75 mg/kg bw	0.66 ± 0.08	1.12 ± 0.04	0.5 ± 0.15	0.52 ± 0.19	0.54 ± 0.09
	150 mg/kg bw	0.86 ± 0.26*** (63)[a]	1.06 ± 0.35	0.54 ± 0.14	0.59 ± 0.18* (30)[a]	0.54 ± 0.04
	800 mg/kg bw	0.99 ± 0.14*** (88)[a]	0.93 ± 0.21	0.54 ± 0.09	0.69 ± 0.15*** (53)[a]	0.59 ± 0.13
		20.8[b]	1.591[b]	1.355[b]	13.31[b]	0.3139[b]

** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.01$.
*** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.001$.
* Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.05$.
[a] Percentage change over control in parenthesis.
[b] F (continuous probability distribution; DFn = 4, DFd = 25).

With respect to DA, DOPAC and HVA levels and turnover in brain regions (see table below), glyphosate at doses of 75, 150 and 800 mg/kg bw produced, in a dose-dependent manner, a statistically significant decrease of DA levels in prefrontal cortex and midbrain compared to control groups. Likewise, glyphosate at the highest dose (800 mg/kg bw) decreased significantly the DA levels in hypothalamus, striatum and hippocampus compared to control group. Moreover, glyphosate at doses of 150 and 800 mg/kg bw decreased significantly the DOPAC metabolite levels in hypothalamus and glyphosate at highest dose only produced a significant decrease of DOPAC levels in hippocampus respect to control group. In addition, the HVA levels significantly decreased after doses of glyphosate 75, 150 and 800 mg/kg bw in hypothalamus, after doses of glyphosate 150 and 800 mg/kg bw in midbrain; and after dose of 800 mg/kg bw in prefrontal cortex respect to control groups. Glyphosate (75, 150 and 800 mg/kg bw) produced a significant increase of the turnover (DOPAC+HVA/DA) in prefrontal cortex respect to control groups. Glyphosate at highest dose only produced a significant increase of the turnover (DOPAC+HVA/DA) in hippocampus (see table below).

Table B.6.7.3.2-3: Effect of glyphosate on DA, DOPAC and HVA levels and turnover (DOPAC+HVA/DA) in brain regions of male rats

Parameter	Animal groups: oral dose of glyphosate during 6 consecutive days	Brain regions				
		Striatum	Hippocampus	Prefrontal cortex	Hypothalamus	Midbrain
DA (ng/g)	Control	6406.35 ± 502.83	248.48 ± 59.36	209.00 ± 45.43	600.20 ± 35.36	776.95 ± 34.09
	35 mg/kg bw	6332.00 ± 296.00	231.00 ± 34.60	186.00 ± 21.33	581.00 ± 51.76	732.00 ± 20.86
	75 mg/kg bw	5614.83 ± 1040.66	213.55 ± 29.46	109.14 ± 16.61*** (−48)[a]	555.48 ± 102.85	676.48 ± 28.33*** (−13)[a]
	150 mg/kg bw	5563.85 ± 223.28	177.84 ± 39.32	97.28 ± 10.78*** (−53)[a]	528.52 ± 39.21	666.07 ± 29.05*** (−14)[a]
	800 mg/kg bw	5009.86 ± 468.29** (−22)[a]	73.07 ± 67.49*** (−71)[a]	34.99 ± 33.65*** (−83)[a]	480.02 ± 23.93* (−20)[a]	642.72 ± 22.98*** (−17)[a]
		6.044[b]	12.49[b]	36.86[b]	4.001[b]	23.71[b]
DOPAC (ng/g)	Control	691.04 ± 64.66	9.51 ± 1.77	21.43 ± 2.68	93.22 ± 21.18	48.75 ± 13.78
	35 mg/kg bw	689.00 ± 74.87	9.44 ± 1.29	21.00 ± 2.23	85.00 ± 18.34	47.00 ± 8.38
	75 mg/kg bw	688.17 ± 221.53	9.00 ± 1.22	19.19 ± 1.23	68.19 ± 21.13	45.78 ± 9.33
	150 mg/kg bw	689.62 ± 37.04	7.66 ± 1.26	18.46 ± 1.01	57.55 ± 6.11** (−38)[a]	44.81 ± 4.09
	800 mg/kg bw	683.38 ± 72.08	6.66 ± 1.95* (−30)[a]	20.04 ± 0.84	57.26 ± 4.70** (−39)[a]	43.78 ± 1.93
		0.00389[b]	4.005[b]	2.958[b]	6.156[b]	0.3051[b]
HVA (ng/g)	Control	907.87 ± 258.95	18.29 ± 1.95	50.75 ± 8.78	68.91 ± 14.12	71.46 ± 3.82
	35 mg/kg bw	906.00 ± 132.32	18.00 ± 3.48	50.60 ± 4.97	64.00 ± 8.34	68.00 ± 5.32
	75 mg/kg bw	901.87 ± 260.40	17.45 ± 4.11	50.33 ± 6.29	44.34 ± 17.40** (−36)[a]	65.10 ± 4.41
	150 mg/kg bw	846.17 ± 48.38	16.17 ± 1.26	46.38 ± 4.27	34.77 ± 5.77*** (−50)[a]	52.91 ± 6.04*** (−26)[a]
	800 mg/kg bw	839.75 ± 32.86	16.40 ± 2.59	30.84 ± 11.55** (−39)[a]	36.82 ± 6.41*** (−47)[a]	38.09 ± 11.10*** (−47)[a]
		0.226[b]	0.6519[b]	7.480[b]	11.50[b]	25.26[b]
(DOPAC+HVA)/DA	Control	0.25 ± 0.05	0.12 ± 0.02	0.36 ± 0.09	0.28 ± 0.25	0.15 ± 0.02
	35 mg/kg bw	0.25 ± 0.04	0.12 ± 0.01	0.39 ± 0.02	0.27 ± 0.06	0.16 ± 0.02
	75 mg/kg bw	0.3 ± 0.11	0.13 ± 0.03	0.64 ± 0.07** (78)[a]	0.22 ± 0.07	0.16 ± 0.02
	150 mg/kg bw	0.28 ± 0.02	0.14 ± 0.03	0.67 ± 0.07** (86)[a]	0.18 ± 0.02	0.15 ± 0.01
	800 mg/kg bw	0.31 ± 0.04	0.98 ± 0.91*** (150)[a]	2.88 ± 2.62*** (153)[a]	0.20 ± 0.02	0.13 ± 0.02
		1.269[b]	5.254[b]	39.28[b]	2.009[b]	2.647[b]

*** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at P < 0.001.
** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at P < 0.01.
* Results are presented are means ± SD from six animals in each group and are significantly different from the control value at P < 0.05.
[a] Percentage change over control in parenthesis.
[b] F (continuous probability distribution; DFn = 4, DFd = 25).

In relation to NE and MHPG levels and turnover, glyphosate at doses of 75, 150 and 800 mg/kg bw produced a significant decrease of the NE levels in striatum and midbrain compared to control. Moreover, glyphosate at doses of 150 and 800 mg/kg bw produced a significant decrease of NE levels in hippocampus and only at a dose of 800 mg/kg bw in prefrontal cortex respect to control groups. Moreover, MHPG levels significantly decreased after doses of glyphosate 150 and 800 mg/kg bw in hippocampus and after dose of glyphosate 800 mg/kg bw in striatum, prefrontal cortex, hypothalamus

and midbrain. Glyphosate at dose of 800 mg/kg bw produced a significant decrease of the turnover (MHPG/NE) in prefrontal cortex and hypothalamus compared to control group (see table below).

Table B.6.7.3.2-4: Effect of glyphosate on NE and MHPG levels and turnover (MHPG/NE) in brain regions of male rats

Parameter	Animal groups: oral dose of glyphosate during 6 consecutive days	Brain regions				
		Striatum	Hippocampus	Prefrontal cortex	Hypothalamus	Midbrain
NE (ng/g)	Control	210.64 ± 79.72	190.74 ± 13.01	145.81 ± 3.08	1006.77 ± 195.00	452.04 ± 76.01
	35 mg/kg bw	202.00 ± 37.93	183.00 ± 11.02	145.00 ± 9.34	1005.00 ± 74.85	422.00 ± 39.44
	75 mg/kg bw	121.07 ± 42.09* (−43)[a]	159.24 ± 20.21	138.54 ± 11.18	1000.88 ± 76.64	357.03 ± 55.11* (−21)[a]
	150 mg/kg bw	118.13 ± 24.65* (−44)[a]	150.02 ± 30.98* (−21)[a]	137.29 ± 6.11	901.07 ± 72.69	358.61 ± 31.77* (−21)[a]
	800 mg/kg bw	102.74 ± 27.10** (−51)[a]	137.34 ± 15.13** (−28)[a]	127.64 ± 12.35* (−12)[a]	878.22 ± 71.54	358.92 ± 17.12* (−21)[a]
		7.194[b]	7.981[b]	3.908[b]	2.007[b]	5.083[b]
MHPG (ng/g)	Control	161.88 ± 21.25	67.07 ± 8.13	109.60 ± 28.56	50.29 ± 8.27	71.69 ± 15.56
	35 mg/kg bw	159.00 ± 35.00	61.00 ± 5.85	100.00 ± 12.67	46.00 ± 11.27	70.00 ± 9.32
	75 mg/kg bw	155.46 ± 36.00	54.90 ± 6.67	84.67 ± 12.91	43.40 ± 7.21	69.11 ± 8.54
	150 mg/kg bw	132.94 ± 31.34	51.22 ± 9.28* (−24)[a]	84.46 ± 12.41	43.03 ± 21.58	67.72 ± 10.57
	800 mg/kg bw	108.95 ± 10* (−33)[a]	50.47 ± 9.85* (−25)[a]	71.11 ± 10.32** (−35)[a]	20.22 ± 9.25** (−60)[a]	29.01 ± 4.66*** (−60)[a]
		3.748[b]	4.451[b]	4.810[b]	5.198[b]	18.61[b]
MHPG / NE	Control	0.85 ± 0.29	0.35 ± 0.06	0.77 ± 0.18	0.05 ± 0.01	0.16 ± 0.03
	35 mg/kg bw	0.84 ± 0.33	0.34 ± 0.05	0.69 ± 0.09	0.05 ± 0.02	0.17 ± 0.02
	75 mg/kg bw	1.42 ± 0.59	0.35 ± 0.08	0.61 ± 0.08	0.04 ± 0.01	0.20 ± 0.04
	150 mg/kg bw	1.14 ± 0.23	0.35 ± 0.08	0.62 ± 0.10	0.05 ± 0.03	0.19 ± 0.03
	800 mg/kg bw	1.10 ± 0.19	0.37 ± 0.07	0.56 ± 0.05* (−23)[a]	0.02 ± 0.01** (−55)[a]	0.18 ± 0.01
		2.733[b]	0.1513[b]	2.792[b]	5.423[b]	1.923[b]

* Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.05$.
** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.01$.
*** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.001$.
[a] Percentage change over control in parenthesis.
[b] F (continuous probability distribution; DFn = 4, DFd = 25).

Discussion

Pesticides are widely used in agricultural and other settings, resulting in continuing human exposure. The nervous system represents a prime target for both the acute and chronic effects of pesticides. Among them are the organochlorines, pyrethroids, organophosphates, neonicotinoids, herbicides and some novel agents. Acute symptoms can include headache, nausea, dizziness, and sensory paraesthesia. Toxicity often involves neuronal hyper-excitability, and disorders of cognition. Toxicological in vitro and in vivo studies have demonstrated specific neurodegenerative effects from exposure to certain pesticides, and human case reports have suggested a causal relationship between certain pesticide exposures and Parkinson's disease (PD) typically associated with degeneration of the dopaminergic neurons. Evidence is also now accumulating that organophosphate pesticides target serotonin and noradrenergic systems contributing to adverse outcomes related to emotional and social behaviours. Ingestion of the herbicide glyphosate may cause significant toxicity including nausea, vomiting, diarrhea, oral and abdominal pain, renal and hepatic impairment, and pulmonary oedema. Impaired consciousness and seizures have also been reported as sequelae but there are limited data of glyphosate on central nervous system (CNS) toxicity. This study was designed to investigate the effects of glyphosate on CNS monoaminergic neurotransmitter contents (5-HT, DA and NE) in male Wistar rats in order to generate more data on the glyphosate neurotoxicity.

The current study showed that exposure to glyphosate, in a region and dose-dependent manner, was accompanied by a significant decrease in the 5-HT, DA and NE contents in the brain regions studied (striatum, hippocampus, prefrontal cortex, hypothalamus, hypothalamus and midbrain), which indicated that glyphosate transfer across the blood-brain barrier, enters the brain, probably accumulates in significant quantity, and exerts neurotoxicity altering the serotonergic, dopaminergic and noradrenergic systems. Researchers have reported similar changes of these brain neurotransmitters after exposure to the insecticide pyrethroid cyfluthrin. It should be noted that the rats treated with glyphosate at dose of 35 mg/kg bw per day did not exhibit any effects on the 5-HT, DA and NE contents in the brain regions studied. In our study, this NOAEL observed (35 mg/kg bw per day) was lowest to that identified on the maternal and developmental toxicity studies (NOAEL of 50 mg/kg bw per day) and used to establish the ADI. For current regulatory evaluation of risks associated with glyphosate exposure, a NOAEL of 35 mg/kg bw per day could be used instead of a NOAEL of 50 mg/kg bw per day. In this regard, taking into account that glyphosate probably accumulates in the

CNS and considering that in the present study the glyphosate exposure was only during only 6 days, further research with a longer period of exposure should be necessary to corroborate the proposed NOAEL of 35 mg/kg bw per day.
In the present study, quantitative analysis of 5-HT, DA and NE contents showed that the loss of these neurotransmitters was mainly observed in the striatum [5-HT and NE contents decreased significantly (-49 % and -51 %, respectively) after the highest dose of 800 mg glyphosate/kg bw] and in prefrontal cortex and hippocampus [DA contents decreased significantly (-83 % and -71 %, respectively) after the highest dose of 800 mg glyphosate/kg bw]. Previous studies also showed in rats that glyphosate decreased DA but not 5-HT levels in striatum as well as reduced the locomotor activity suggesting that the decrease in striatal DA levels could also explain a behavioural hypoactivity. Moreover, in our study, glyphosate after the highest dose of 800 mg/kg bw produced a significant increase of the (5-HIAA/5-HT) turnover in striatum (88 %) and of the (DOPAC+HVA/DA) turnover in prefrontal (153 %) and hippocampus (150 %), but a significant decrease of the (NE/MHPG) turnover in prefrontal cortex (-23 %) and hypothalamus (-55 %), critical brain regions that regulate cognitive functions. The cooperation of the hippocampus and the prefrontal cortex is vital in spatial working memory performance and decision making. Disconnection or damage to either of the two brain regions induces impaired cognitive behaviours. Because cognitive functions are quite complex, more details are required for the complete understanding of glyphosate-induced neurotoxicity. It would be of interest to investigate the developmental changes of the hippocampus and prefrontal cortex in prenatal glyphosate.

III. CONCLUSION

The authors concluded that the results demonstrate that glyphosate leads to loss of 5-HT, DA and NE levels in the CNS. The neurochemical effects observed in the present study are an important public health concern. Although there was no data on humans, glyphosate could exert its neurotoxicity, notably on monoamine systems, by inducing DNA damage, neuronal inflammation and oxidative stress mechanisms. Further investigation is needed to involve the glyphosate herbicide with neurodegenerative diseases.

Assessment and conclusion by applicant:

Although the study concludes “loss of 5-HT, DA and NE levels in the CNS”, no historical controls are available to assess and compare the changes in the treatment-groups to ascertain if the effects are within background or if they are biologically relevant.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because there were no negative or positive historical control data to establish whether changes in the levels of neurotransmitters were biologically meaningful. No concurrent positive control was included to demonstrate assay viability. Also no analytical verification of dose levels are available.

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

Publication: Martínez, M. *et al.*, 2018	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Non-guideline
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Technical glyphosate purity of ≥98 %. Source: Sigma-Aldrich. No information on

		storage,
Only glyphosate acid or one of its salts is the tested substance	Y	Yes
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	Wistar rat (male)
Test conditions clearly and completely described	Y	Yes
Route and mode of administration described	Y	Oral by gavage.
Dose levels reported	Y	35, 75, 150, 800 mg/kg bw/day for 6 days
Positive control	N	-
Number of animals used per dose level reported	Y	6/dose group.
Method of analysis described for analysis test media	N	-
Validation of the analytical method	N	-
Analytical verifications of test media	N	-
Complete reporting of effects observed	N	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	No positive or negative historical control data and no concurrent positive control data included, no analytical verification of dose or stability.
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because there was no historical control to determine if changes in the levels of neurotransmitters were within historical controls. No positive control was included. Also no analytical verification of dose levels are available.		

Assessment and conclusion by RMS:

The study adequately describes the key elements of the Material and Methods and Results section. However, as also indicated by the applicant no negative or positive historical control data was provided to establish whether changes in the levels of neurotransmitters were biologically relevant and no concurrent positive control was used to determine study performance. Overall, the study is concluded to be reliable with restrictions.

B.6.7.3.3. Publications on neurotoxicity – study 3

Data point:	CA 5.7/003
Report author	Chorfa, A. *et al*.
Report year	2013
Report title	Specific pesticide-dependent increases in α-synuclein levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines.
Document No	doi:10.1093/toxsci/kft076 E-ISSN: 1096-0929.
Guidelines followed in study	None
Deviations from current test guideline	Not applicable

Previous evaluation	Yes, evaluated in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Study reliable.

1. Full summary of the study according to OECD format

The objective was to precisely assess changes in α-syn levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines following acute exposure to several pesticides including glyphosate using Western blot and flow cytometry. The study was conducted using an in vitro test system. Glyphosate did not have any impact on the endpoints measured in this study.

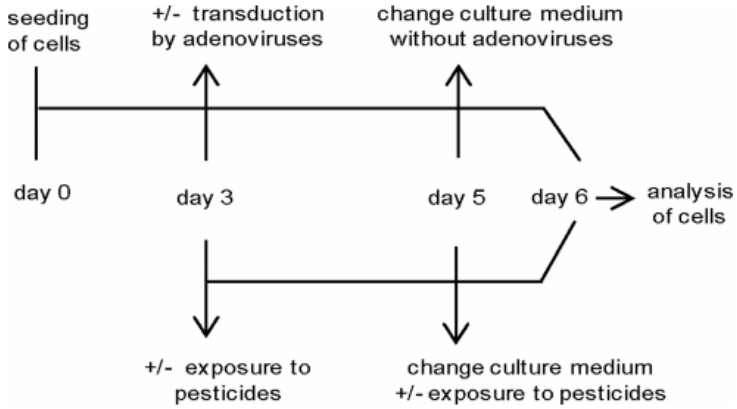
I. MATERIALS AND METHODS

Cell culture: SH-SY5Y (a human dopaminergic neuroblastoma cell line) and SK-MEL-2 (a human cutaneous melanoma cell line) obtained from American Type Culture Collection (ATCC, Rockville, MD) were maintained in Dulbecco's modified Eagle's (DMEM-F12-GlutamaxI) medium containing 10 % fetal bovine serum (Invitrogen), 100 U/mL penicillin, and 100 µg/mL streptomycin in an incubator at 37 °C and 5 % CO2.

Recombinant AdV-mediated overexpression of α-syn. Cell transduction was performed as previously described (see study report). Briefly, a recombinant adenoviral genome containing the full-length complementary DNA encoding human WT and mutant A53T α-syn in frame with a C-terminal myc-His epitope tag was generated by homologous recombination. Cells were infected with α-syn AdV or GFP AdV. On day 2 of infection, the medium was replaced with AdV-free DMEM.

Pesticides exposure: Cells at 70 % confluence were exposed to the pesticides, including glyphosate (N-(phosphonomethyl)-glycine) (Sigma-Aldrich). Purity was 99.5 %. Glyphosate was dissolved in ultrapure water. Glyphosate concentrations for cell exposures (at 75 and 50% viability) were chosen based on the evaluation of toxicity following exposure of the SH-SY5Y cell line (0.005-800 µM) using the 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The protocol used for cell exposure and / or transduction with recombinant AdVs is shown in the figure below.

Figure B.6.7.3.3-1: Experimental protocol of exposure to pesticides and recombinant AdV transduction of SH-SY5Y neuroblastoma and SK-MEL-2 melanoma cell lines.



At approximately 70 % cell confluence (day 3), cells were transduced with recombinant AdVs for protein overexpression (WT α-syn, A53T α-syn, GFP) and/or exposed to pesticides (rotenone, paraquat, maneb, and glyphosate) at concentrations corresponding to the IC50 determined on the SH-SY5Y cell line. For certain experiments, these two steps (pesticides exposure/adenoviral transduction) were combined. After 48 h (day 5), two protocols were followed. When the cells were only transduced with AdV, the culture medium was replaced by fresh AdV-free culture medium. When the cells were exposed to

pesticides, the culture medium was replaced by fresh AdV-free culture medium supplemented with pesticides. Experiments were ended 24 h later (day 6), and adherent cells were collected for analyses by flow cytometry or Western blot.

Cell death and viability assays: The MTT assay was performed with the Celltiter96 nonradioactive kit (Promega, France). MTT is metabolically converted into formazan by mitochondrial dehydrogenases of healthy, living cells. Briefly, 5×10^4 cells per well were seeded into 96-well plates in duplicate and then treated with different concentrations of glyphosate. Cell death was assessed after 72 h of pesticide exposure. Then, 15 µL of "dye solution" was added to each well, and the plates were incubated at 37 °C, 5 % CO_2, for 4 h. Finally, 100 µL of "solubilization/stop solution" was added. After incubation for 1 h at 37 °C, the optical density of the dissolved formazan grains within the cells was measured spectrophotometrically at 560 nm (BioTek ELx808, France). Results were expressed as percentage of the control. The IC_{50}, half-maximal (50 %) inhibitory concentration, was determined for each pesticide from the graph of cell viability.

Protein extraction and Western blotting: After pesticide exposure, the cells were harvested and lysed in Laemmli buffer and then heated for 10 min at 100 °C. The cell extract was then centrifuged (15,000 × g, 30 min at 4 °C) before being loaded on to 12 % gel for SDS-PAGE. Western blots were performed as previously described (see study report). Blots were hybridized with monoclonal antibodies against β-actin (Abcam, dilution 1:1000) and against α-syn (clone 42, BD Biosciences, dilution 1:2000) overnight at 4 °C. The membranes were then washed and further hybridized with goat anti-mouse immunoglobulin G (IgG) horseradish peroxidase–conjugated antibody (Pierce, dilution 1:1000) for 30 min at room temperature. Protein bands were detected with chemiluminescent reagents (SuperSignal West Dura Extended Duration Substrate Kit, Pierce), then exposed to autoradiographic films or to a CCD camera (Versadoc system 5000, Bio-Rad), and quantified by Quantity One software (Bio-rad).

Flow cytometry: The cells were harvested by centrifugation at 1000 × g for 5 min at 4 °C. The cell pellets were resuspended in a blocking solution (2 % BSA-PBS) at 4 °C. The cells were simultaneously permeabilized and fixed for 20 min at 4 °C with BD Cytofix/Cytoperm Kit (BD Biosciences). All steps were carried out with permwash solution (BD Permwash Kit). After another centrifugation step, the cell pellets were incubated with α-syn clone 42 antibody in permwash solution for 30 min at 4 °C. They were then rinsed with permwash solution and incubated with goat anti-mouse IgG R-phycoerythrin conjugate (Invitrogen) at 4 °C for 30 min. The specific fluorescence intensities were measured with a BD FACS LSRII analyzer (BD Biosciences). Data were acquired using Diva software (BD Biosciences) and analyzed with FlowJo software (v7.6.5-TreeStar, Ashland, Oregon).

Statistical analysis: The results represent means ± SEM from at least 3 and up to 15 independent experiments. The effects of glyphosate on cell survival, following exposures of the four cell types (SH-SY5Y ± WT α-syn AdV and ± A53T α-syn AdV and ± GFP AdV) to different concentrations, were determined from an analysis of covariance. The results from Western blot studies were subjected to a Wilcoxon's test. For the flow cytometry studies of AdV-transduced cells, the mean fluorescence intensities of cells exposed or not exposed to glyphosate were compared using Student's test. Then, we realized a Levene's analysis in order to verify the hypothesis of homogeneity of variances. If the hypothesis was not verified, the Welch's analysis was used.

II. RESULTS

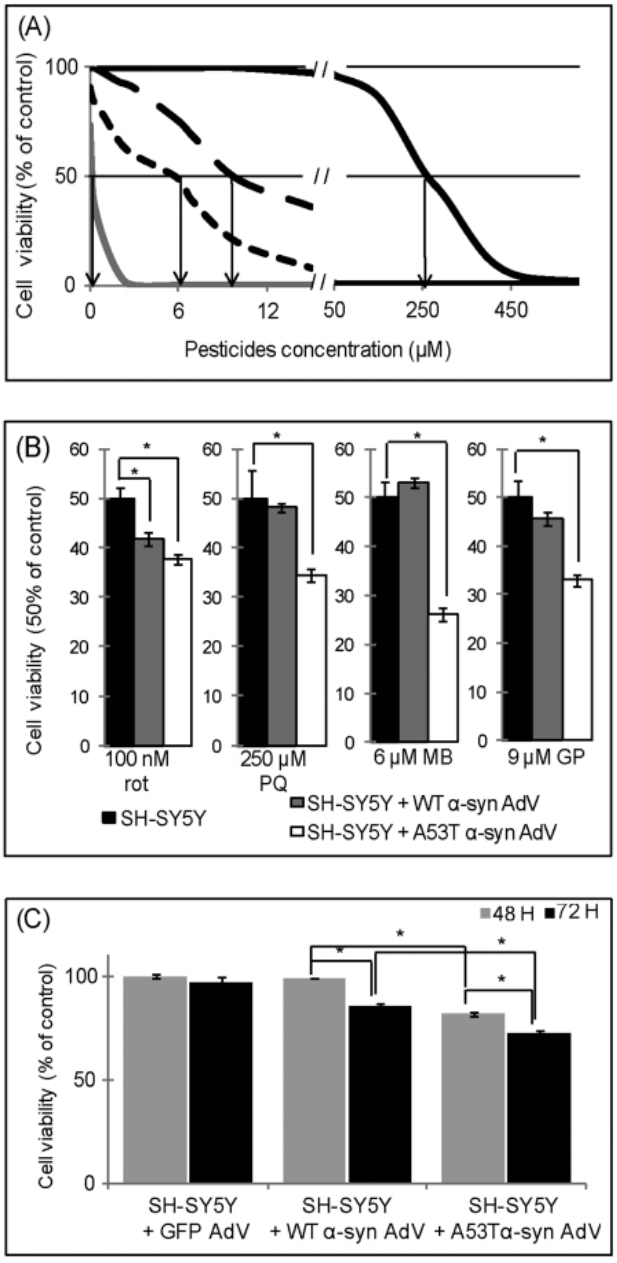
The impact of in vitro exposure to glyphosate on α-syn levels was assessed in two human cell lines of neuronal (SH-SY5Y) or melanocytic (SK-MEL-2) origin. The levels of endogenous α-syn or of recombinant α-syn levels after transduction with recombinant AdVs (WT and A53T α-syn AdV) were analyzed by Western blot and flow cytometry.

Cytotoxicity Associated With Pesticide Exposure and/or Adenoviral Transduction

The SH-SY5Y neuronal cell line was first exposed to various concentrations of glyphosate (0.005-800 µM) for 72 h. The respective cytotoxicity of glyphosate (relative amounts of living and

metabolically active cells) was estimated by MTT assay (see figure below). This showed that glyphosate had a distinct effect on the survival of SH-SY5Y cells after 72 h of exposure. The half-maximal (50%) inhibitory concentrations (IC50) for glyphosate was 9 µM. It was 11 µM for glyphosate for the SK-MEL-2 cell line. The IC50 value, determined after exposure of the SH-SY5Y cell line for 72 h, was chosen for further investigations of the effects on α-syn levels. The same concentration was used in experiments with the SK-MEL-2 melanoma cell line.

Figure B.6.7.3.32: Effects of pesticides and adenoviral transduction on viability of SH-SY5Y neuroblastoma cells.



(A) Cell viability was assessed using the MTT assay. Data represent mean ± SEM numbers of viable treated cells/numbers of viable untreated cells from three separate experiments (*$p < 0.05$). The p values were determined by Wald's test. The cell viability percentage after 72-h treatment with increasing concentrations (0.005-800 µM) of pesticide was measured: rotenone (grey curve), maneb (dashed black curve), glyphosate (black curve em dash), or paraquat (black solid curve).

(B) Part of the concentration range has been removed to facilitate comparison of the results. We then used the IC_{50} previously determined on SH-SY5Y cells (100 nM and 6, 9, and 250 µM) to quantify the viability of cells transduced with WT or A53T α-syn AdV after pesticide exposure for 72 h in comparison to untransduced SH-SY5Y cells.

(C) The cytotoxicities associated with the recombinant Advs transduction alone, with either the GFP protein, WT or A53T α-syn AdV, were measured at 48 h (grey bars) and 72 h (black bars).

Glyphosate cytotoxicity, following exposure for 72 h of SH-SY5Y cells transduced with recombinant AdVs, was then analyzed using the protocol summarized in Figure B.6.7.3.3-1. The MTT assay did not reveal a significant decrease in the viability of WT α- syn AdV-transduced cells after exposure to glyphosate [-2 % [p = 0.6]) at the previously determined IC50 (see Figure B.6.7.3.3-2 (B)). Viability was significantly decreased (-17%) with glyphosate ($p < 0.01$) when the cells were transduced with A53T α-syn AdV. Thus, glyphosate induced a greater reduction of viability in cells transduced with A53T α-syn AdV than in nontransduced SH-SY5Y cells.

It should be noted, however, that viability was also consistently decreased in the absence of pesticide exposure, after transduction with WT α-syn AdV (-14 %)($p < 0.05$) and even more so with A53T α-syn AdV (-38 %)($p < 0.05$) at 72 h, as shown in Figure B.6.7.3.3-2 (C) representing three different experiments. Viability was already decreased 48 h after transduction with A53T α-syn AdV (-18%) ($p < 0.05$). Cytotoxicity seemed to be related to α-syn overexpression, as viability was unchanged 48 or 72 h after transduction with the GFP AdV used as a control in these experiments.

Specific Increase of Endogenous α-syn in Human Neuroblastoma and Melanoma Cells Exposed to Pesticides

Both SH-SY5Y neuroblastoma and SK-MEL-2 melanoma cell lines express α-syn. We therefore assessed endogenous α-syn expression using Western blot and flow cytometry and examined the ability of glyphosate exposure to modulate changes in α-syn levels. The Western blot experiments indicated that endogenous α-syn levels (B.6.7.3.3-3 A) were significantly increased by 100 nM rotenone (~1.81×) ($p < 0.001$), whereas no change was observed in the closely related, but nonamyloidogenic, β-synuclein protein B.6.7.3.3-3 C). The impacts of glyphosate on the level of endogenous α-syn in SH-SY5Y neuroblastoma cells was measured in comparison (B.6.7.3.3-3 A). A significant increase in α-syn levels was not observed with glyphosate up to 9 µM ($p = 0.6553$).

Quantification of Recombinant α-Syn Levels by Flow Cytometry Following Pesticide Exposure

We then attempted to quantify the changes in α-syn levels following transduction with AdV designed to overexpress α- syn. Transduction of SH-SY5Y cells with WT or A53T α-syn AdV resulted in similar levels of α-syn expression as measured by flow cytometry. In comparison to the isotype control, a 5.1 % increase in fluorescence intensity was observed compared with 1.7% for the endogenous protein. Similar levels of α-syn were produced in α-syn AdV-transduced SK-MEL-2 cells (data not shown). The Western blot revealed that transduction with recombinant α-syn AdV was associated with a predominant band at 22.5 kDa, resulting from the presence of the myc-His tag epitope, in addition to the 19 kDa band representing the endogenous α-syn.

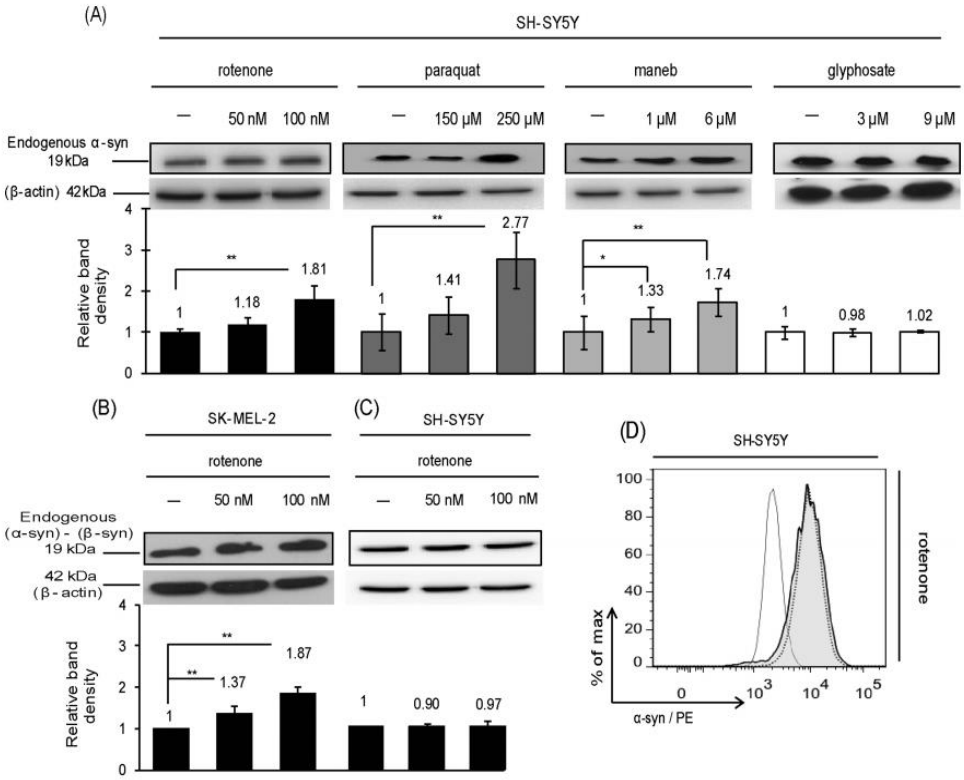
The authors then confirmed the effects of pesticides on AdV-associated α-syn levels by Western blot (Figure B.6.7.3.3-4). In AdV-transduced SH-SY5Y cells, 1.38- and 1.70-fold increases were observed with WT (left panel) and A53T (right panel) α-syn, respectively, following exposure to 100 nM rotenone ($p < 0.001$).

Pesticide impact was then assessed by flow cytometry after 72-h exposure of SH-SY5Y cells transduced with WT or A53T α-syn AdV to the IC_{50} (determined on the SH-SY5Y cell line) in comparison to a GFP AdV control (Figure B.6.7.3.3.-5). The observed increases in α-syn levels were specific, as no change in GFP fluorescence was apparent after exposure to glyphosate (data not shown) of cells transduced with a GFP AdV control.
No increase in α-syn levels was detected after exposure of SH-SY5Y cells transduced with WT or A53T α-syn AdV to glyphosate.

No significant increase in α-syn levels was found after the exposure to glyphosate of SK-MEL-2 cells transduced with WT or A53T α-syn AdV (compared with those transduced with GFP AdV).

Figure B.6.7.3.3-3 - 3: Characterization of the impact of pesticides on endogenous α-syn levels in

SH-SY5Y and SK-MEL-2 cells by Western blot and flow cytometry.



(A) The levels of endogenous α-syn were estimated by Western blot after exposure of SH-SY5Y cells to different pesticides at concentrations corresponding to 25 and 50 % of the IC_{50} (50 and 100 nM rotenone; 150 and 250 µM paraquat; 1 and 6 µM maneb; 3 and 9 µM glyphosate).

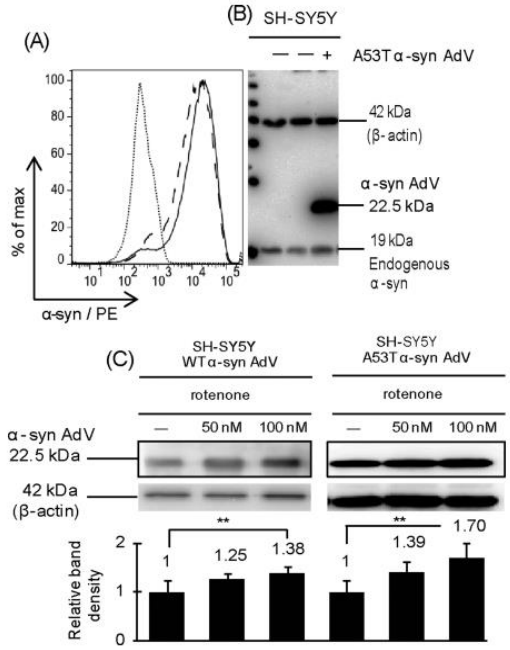
(B) Similarly, the levels of endogenous α-syn in SK-MEL-2 cells exposed to 50 and 100 nM rotenone were also assessed.

(C) In parallel, we estimated the amounts of β-synuclein in SH-SY5Y cells after rotenone exposure.

(D) β-actin was used as a loading control. Data represent the means ± SEM from four to six independent experiments obtained by Wilcoxon's test (**p < 0.001; *p < 0.05). The fold increase is indicated above the histogram for each experimental condition. Finally, the fluorescence curves corresponding to the levels of endogenous α-syn (dotted curve) in SH-SY5Y cells observed by flow cytometry after exposure to 100 nM rotenone (black curve, shaded area) are compared with SH-SY5Y cells immunostained with an isotype control (grey curve)(D).

Data represent the means ± SEM from three independent experiments.

Figure B.6.7.3.3-4 **- 4: Characterization of the impact of pesticides on recombinant α-syn levels in SH-SY5Y cells by Western blot and flow cytometry.**



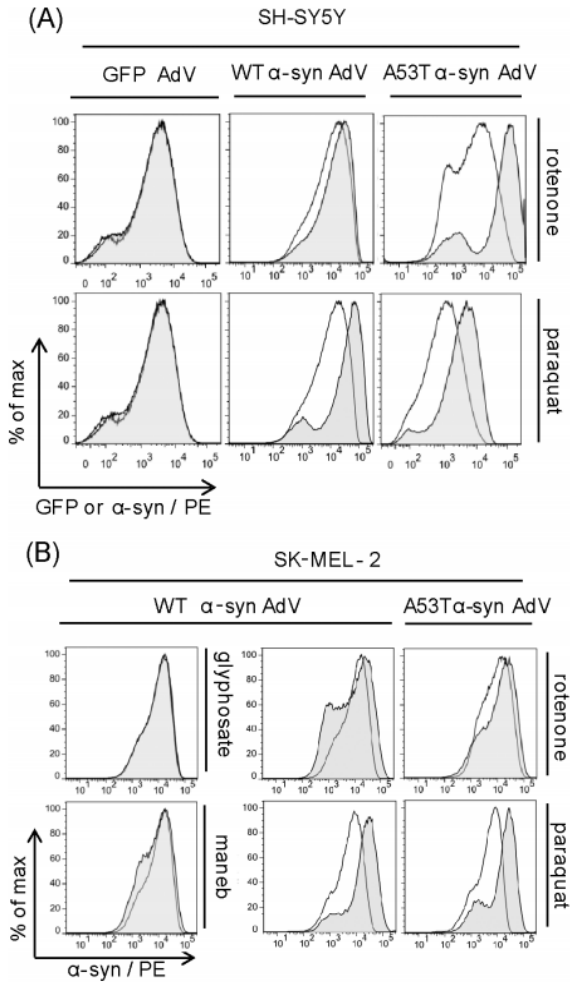
(A)Three days after adenoviral transductions, the cells were permeabilized and immunostained with clone 42 antibody raised against α-syn. The flow cytometry histogram shows the levels of recombinant α-syn in SH-SY5Y cells overexpressing either WT (dashed line) or A53T (solid line) α-syn AdV, in comparison to endogenous α-syn (grey line).

(B) Western blot detection of recombinant α-syn in comparison to endogenous α-syn is shown.

(C) Finally, the levels of recombinant α-syn were quantified in SH-SY5Y cells transduced with either WT or A53T α-syn AdV following rotenone exposure at 50 and 100nM.

Data represent the mean ± SEM α-syn/β-actin ratios from four independent experiments (**$p < 0.001$ obtained by Wilcoxon's test).

Figure B.6.7.3.3-4 - 5: Characterization of the impact of pesticides on α-syn levels in AdV-transduced SH-SY5Y and SK-MEL-2 cells by flow cytometry.



(A) Fluorescence curves correspond to the α-syn levels following AdV transduction of SH-SY5Y cells by GFP AdV (left column, solid curve not shaded), WT α-syn AdV (center column, solid curve not shaded), and A53T α-syn AdV (right column, solid curve not shaded) alone or following exposure to either 100nM rotenone (upper panel, solid curve, grey shaded) or 250µM paraquat (lower panel, solid curve, grey shaded).

(B) Fluorescence curves correspond to the α-syn levels in SK-MEL-2 after transduction with WT α-syn AdV (left and center column, solid curve not shaded) or A53T α-syn AdV (right column, solid curve not shaded) alone or following exposure to 9µM glyphosate (solid curve, grey shaded), 6µM maneb (solid curve, grey shaded), 100nM rotenone (upper panel, solid curve, grey shaded), or 250µM paraquat (lower panel, solid curve, grey shaded).

Data represent the means ± SEM of 6 to 12 separate experiments for each of the different pesticide exposures.

III. DISCUSSION & CONCLUSION

Overall, the specific effects of the pesticides were quite consistent for both endogenous and AdV-produced α-syn. The mechanisms involved in the pesticide-induced increases were not examined but could reflect a decreased efficiency of the cellular mechanisms involved in the degradation of misfolded proteins such as the proteasome pathway, and/or, for the endogenous protein, increased synthesis of the protein. The changes in protein levels were specific to α-syn, as no changes were observed for a GFP protein encoded by recombinant AdVs or for the endogenous β-synuclein expressed in the SH-SY5Y cell line. β-Synuclein shares a high homology sequence with α-syn but is less prone to aggregation in relation with the absence of 11 amino acids in the central region of the protein.

No effect of the pesticide glyphosate on α-syn levels was detected. Although a case of parkinsonian

syndrome was reported following acute poisoning with glyphosate (Barbosa et al. 2001 (refer to B6.9.8.15)), it was concluded in a new study that there is little evidence to suggest a causal relationship between glyphosate exposure and noncancer diseases, including PD.

It was found that paraquat, and to a lesser extent rotenone, but not maneb or glyphosate, also increased the α-syn levels in SK-MEL-2 cells, whether produced endogenously or after adenoviral transduction. In summary, we provide an approach based on commonly used methods, which allow the quantification of cellular α-syn levels, and we show here that these levels are greatly increased following exposure to certain pesticides, which have been specifically associated with PD. This experimental strategy could provide useful and readily available information specific to each pesticide so that the neurotoxic potential of the chemical can be assessed prior to large scale use in the field. On this basis, further studies are now focusing on the development of analytical methods in microplate format, which would be more convenient for large scale screening. To what extent such in vitro observations reflect specific events involved in the molecular pathogenesis of human α-syn associated diseases remains to be determined.

1. Assessment and conclusion

Assessment and conclusion by applicant:

The objective was to precisely assess changes in α-syn levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines following acute exposure to glyphosate using Western blot and flow cytometry. The study was conducted using an *in vitro* test system. Glyphosate did not have any impact on the endpoints measured in this study. This is not a guideline study, nor did this study evaluate an endpoint used in risk assessment. Therefore, this study in not usable for quantitative human health risk assessment or hazard assessment.

This publication is considered reliable with restrictions (no positive control was included and only 2 test concentrations were used) but is not relevant for the risk assessment of glyphosate.

Reliability criteria for *in vitro* toxicology studies

Publication: Chorfa *et al*., 2013	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 99.5 %. Source: Sigma-Aldrich.
Only glyphosate acid or one of its salts is the tested substance	N	Also other pesticides tested (rotenone, paraquat, maneb).
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0, 3, and 9 µM.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive controls

		used.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered not relevant for the risk assessment of glyphosate because it is not a guideline study, nor did this study evaluate an endpoint used in risk assessment. It is reliable with restrictions because no positive control was included and only 2 test concentrations were used.		

Assessment and conclusion by RMS:

The study adequately described the key elements of the Materials and Methods section and Results section. As indicated by the applicant no positive control was included. However, positive results were obtained with paraquat and therefore this is not considered to be a limitation by the RMS. Overall, the study is concluded to be reliable (Klimisch Score 1).

B.6.7.3.4. Publications on neurotoxicity – study 4

Data point	CA 5.6 (Note AGG: the applicant submitted this publication for section B.6.6.1 (generational studies) but it is considered more appropriate for the neurotoxicity section (B.6.7) of the RAR)
Report author	Ait-Bali Y. *et al.*
Report year	2020
Report title	Pre- and postnatal exposure to glyphosate-based herbicide causes behavioral and cognitive impairments in adult mice: evidence of cortical ad hippocampal dysfunction.
Document source	Archives of toxicology, (2020) Vol. 94, No. 5, pp. 1703-1723
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability	Supplementary

Aim of the study

The present study was conducted using a multifaceted behavioral battery to assess the profile of gestational and lactational GBH effects in a mouse model. Behavioural assays covering neonatal age and adulthood were selected to measure a range of early reflex development as well as locomotor, affective, sociability and cognitive functions. Mechanistic, cytoarchitectural, neurochemical and molecular mechanisms underlying of GBH-induced neurobehavioral deficits were evaluated, with the goal to provide benchmark data for GBH risk assessment in the brain.

Materials and methods

Test material:	Roundup herbicide (glyphosate concentration: 360 g/l as isopropylamine salt 486 g/l), was supplied by Monsanto Company, St. Louis, MO, USA
Lot/Batch:	Not reported
Purity:	Not reported
Vehicle:	Tap water
Test animals:	Mice

Strain:	Swiss
Age/weight on arrival:	3 month/weight not reported
Source:	Faculty of Sciences, Cadi Ayyad University, Marrakech, Morocco
Housing:	Not reported
Acclimatisation period:	Not reported
Diet:	Not specified, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	Temperature 22±2 °C, light/dark cycle 12 h/12 h and humidity not reported

Experimental design
Females were mated with breeding males (two females for one male) over a day and were examined on the following day by vaginal plug inspection to assess successful mating. If judged copulated, the female was removed from the cage of the male and housed individually. This was considered as the day 0 of gestation (G0). Female exposure to GBH through oral gavage occurred daily from G0 to postnatal day 21 (P21). Three experimental groups were formed, each one including a minimum of six female mice: a group exposed to a lower dose (250 mg/kg) of GBH, a group exposed to a higher dose (500 mg/kg) of GBH, and a control group which received vehicle (tap water).

Gestation outcomes, maternal behaviour and body weight of pups
To detect any signs of poisoning, all pregnant mice were observed daily from the first administration day (G0) until parturition. In addition, several parameters of maternal behaviour, fertility and reproduction were evaluated.

Motor and sensory development assessment
Behavioural testing (negative geotaxis, righting reflex, cliff avoidance and rotarod tests) was performed. Animals (n = 10 for each group: control, 250 mg/kg and 500 mg/kg; two males from each litter) were tested during morning sessions starting at 9 a.m. The tests for sensorimotor development assessed during the same day were separated by an interval of 30 min.

Adult behaviour
From P60, behavioral tests were performed to assess locomotor activity (open field, OF), levels of anxiety (OF and elevated plus-maze, EPM), social interaction (three-chambered sociability test, TCS), working memory (Y-maze), recognition memory (novel object recognition test, NOR) and learning and emotional memory performances (passive avoidance test, PA). The behaviour of a total number of ten mice for each group was evaluated between 9 a.m. and 13 p.m. and was recorded.

Determination of acetylcholinesterase enzyme activity
After the behavioural analyses, mice were killed by decapitation, their brains immediately removed from the skull and the PFC and hippocampi were dissected for biochemical analyses. Acetylcholinesterase (AChE) activity was determined in tissue homogenates.

Tissue sampling and immunofluorescence
Upon conclusion of behavioural testing, control and treated mice were anesthetized with an intraperitoneal injection of urethane 40% and transcardially perfused with saline solution, followed by ice-cold 4% formaldehyde in phosphate-buffered saline. The brains were then removed, post-fixed in the same fixative for 12 h and cryoprotected overnight in 30% sucrose. They were then cut on a freezing cryostat into 30 µm frontal sections. The sections containing the substantia nigra pars compacta (SNc), the ventral tegmental area (VTA) and the striatum were used for tyrosine hydroxylase (TH) immunofluorescence, while sections containing PFC and dorsal hippocampus stocked for GFAP and Iba-1 immunofluorescence.

Image analysis
The TH-immunostained sections were used to assess the number of dopaminergic cells in the SNc and VTA and dopaminergic fibers in the striatum, while the GFAP and Iba-1 immunostained sections were used to assess reactive astocytes and microglia, respectively, in the PFC and dorsal hippocampus. Three mice per group were used for these analyses. All analyses were carried out by an operator blinded to the experimental groups.

RNA isolation, cDNA preparation, and quantitative real-time PCR
Twenty-four hours following the completion of behavioural and cognitive tests, RNA was extracted from PFC and hippocampus tissues from control and GBH 500 mg/kg exposed mice (n = 3 each). RNA level was quantified by measuring absorbance at 260 and 280 nm. Following retrotranscription of total RNA, quantitative

real-time PCR (qRT-PCR) was carried out. Analyses were performed in technical duplicate and biological triplicate.

Statistical analysis
Fertility and reproduction parameters as well as maternal behaviour were analysed by one-way ANOVA, while body weight gain and early sensorimotor endpoint results were analysed using the repeated measure two-way ANOVA (GBH dose and age), followed by a Holm-Sidak's post hoc test for multiple comparisons. The dataset of behavioural tests in adult mice, enzyme activity results and histological assays were compared between different groups (treated and control) and analysed using one-way ANOVA, followed by a Holm–Sidak's post hoc for multiple comparisons. The biomolecular data were analysed with t test. The results are presented as mean ± SEM, and a value of $p < 0.05$ was considered statistically significant.

Results

Gestation outcome and maternal behaviour following GBH exposure
Administration of GBH to pregnant females affected fertility and reproduction parameters. Indeed, the fertility rate and the gestational index were lower in treated groups compared to control. Similarly, both the number of litters and the total number of mice per litter were significantly lower in the GBH-exposed groups. Treatment with GBH 500 mg/kg, but not with the lower dosage, also affected retrieving and nesting index (Table 2). In contrast, the statistical analysis did not reveal a significant difference in the gestation length between treated and control groups ($F_{(2\ 18)} = 1.08$, $p > 0.05$) (Table 1).

Table 1*: Reproductive findings in mice given GBH during pregnancy and lactation

	Glyphosate-based herbicide		
	0 (control)	250 mg/kg	500 mg/kg
No. of females copulated	8	10	24
No. of pregnant females	7	6	6
Fecundity index (%)	87	60	25
No. of death during pregnancy	0	0	1
Gestation length (days)	19.8 ± 0.37	20 ± 0.54	19.2 ± 0.2
No. of females with live born	7	6	5
Gestation index (%)	100	100	83
No. of females with totally litter loss	0	0	1
No. of litters	7	6	5
Total no. of pups born	56	40	35
No. of pups born alive	51	34	33
No. of dead pups	5	6	2
Delivery index (%)	100	100	100
Lactation index (%)	88.2	79.4	69.6
Nest building index (%)	100	100	80
Retrieving index (%)	100	100	80
% of males	37	45	40

Fecundity Index (%) = (no. of pregnant females/no. of females copulated) × 100
Values are given as the mean ± SD
Gestation index (%) = (no. of females with live pups born/no. of pregnant females) × 100
Delivery index (%) = (no. of females delivering/no. of pregnant females) × 100
Lactation index (%) = (no. of living offspring on day 21/no. of offspring born alive) × 100
Nesting index = (no. of females building theirs nests for a maximum duration of 30 min/no. of females delivering) × 100
Retrieving index = (no. of females retrieving over pups for a maximum duration of 30 min/no. of females delivering) × 100
% of males = (no. of males/no. of pups) × 100
*Retrived from Ait-Bali, Y., *et al*., Archives of Toxicology (2020) 94: 1703-1723

GBH decreased body weight of pregnant females and their offspring
The two-way repeated measures ANOVA showed a significant effect of treatment ($F_{(2,19)} = 11.80$, $p < 0.01$) and period of treatment ($F_{(2,19)} = 211.62$, $p < 0.001$) on dam weights. However, the interaction between the two

factors was not significant ($F_{(2,19)}$ = 0.95, p > 0.05). The post hoc comparisons showed that body weight gain was significantly reduced following GBH treatment at both 250 and 500 mg/kg with respect to the control (p < 0.05) (Table 2). Likewise, our results indicated a significant effect of treatment and age on pup's body weight ($F_{(2,19)}$ = 11.80, p < 0.01; $F_{(2,19)}$ = 11.80, p < 0.01, respectively) as well as for the interaction between the two factors ($F_{(2,19)}$ = 11.80, p < 0.01). Furthermore, Holm-sidak comparisons revealed a significant reduction of body weight gain in offspring delivered from both GBH exposed dams groups p < 0.05) (Table 2).

Table 2*: GBH affects body weight gain of mice mothers and their offspring

	Glyphosate-based herbicide			250 vs control		Post hoc 500 vs control		250 vs 500	
	0 (control)	250 mg/kg	500 mg/kg	*t*	*p*	*t*	*p*	*t*	*p*
Pregnancy									
G1	0.78 ± 0.34	− 1.22 ± 0.48	− 3.66 ± 0.14	3.59	ns	7.98	***	1.94	ns
G7	5.24 ± 0.55	− 2.20 ± 0.66	0.24 ± 0.49	5.46	*	8.98	***	1.56	ns
G15	10.38 ± 0.67	8.12 ± 0.55	5.18 ± 0.56	4.06	ns	9.34	***	2.34	ns
G19	16.34 ± 0.65	14.16 ± 0.49	10.48 ± 0.78	3.91	ns	10.53	***	2.93	**
Lactation									
L1	9.22 ± 0.95	5.04 ± 1.19	3.80 ± 0.76	4.34	ns	5.63	*	0.98	ns
L7	10.00 ± 0.93	6.76 ± 1.28	5.52 ± 1.01	3.37	ns	4.66	ns	0.33	ns
L15	11.06 ± 0.66	7.54 ± 1.31	6.30 ± 0.83	3.66	ns	4.95	*	2.80	ns
L19	11.44 ± 0.83	8.44 ± 1.01	7.20 ± 0.51	3.12	ns	4.41	ns	0.33	ns
Litter									
P1	1.31 ± 0.02	1.15 ± 0.07	1.05 ± 0.88	1.02	ns	1.65	ns	0.51	ns
P7	2.51 ± 0.03	2.08 ± 0.14	1.91 ± 0.11	2.68	ns	3.79	ns	0.90	ns
P15	5.47 ± 0.23	4.06 ± 0.22	3.17 ± 0.13	8.92	***	14.52	***	4.59	***
P21	7.56 ± 0.18	6.57 ± 0.24	6.43 ± 0.20	6.23	**	7.10	***	0.71	ns

G gestation day, *L* lactation day, *P* postnatal day

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns (no significant) $p > 0.05$

*Retrived from Ait-Bali, Y., *et al*., Archives of Toxicology (2020) 94: 1703-1723

GBH delayed developmental skills of pre- and postnatally exposed offspring

Righting reflex

Investigation whether pre- and postnatal exposure to GBH caused atypical sensorimotor skills development in offspring. Two-way repeated measure ANOVA showed a significant main effect of treatment ($F_{(2,23)}$ = 13.83, p < 0.001) and the expected effect of age, indicating a reduction in righting time as the mice developed ($F_{(2,23)}$ = 309.10, p < 0.001). Notably, GBH treatment delayed the development of the righting reflex as shown by a significant interaction between the two factors ($F_{(2,23)}$ = 8.38, p < 0.001). Post hoc analysis revealed that mice treated with 250 mg/kg or 500 mg/kg GBH had slower righting reflexes time than the controls only at P5 (250 vs control mg/kg: q = 7.45, p < 0.001; 500 mg/kg vs control: q = 10.32, p < 0.001) (Fig.. 1A).

Negative geotaxis

The two-way ANOVA revealed a significant effect of treatment ($F_{(2,23)}$ = 83.84, p < 0.001) and the expected maturation effect reflected by the significant effect of age ($F_{(2,23)}$ = 59.44, p < 0.001). The interaction was also significant ($F_{(2,23)}$ = 23.41, p < 0.001). Post hoc comparisons confirmed that mice treated with 500 mg/kg had higher negative geotaxis time compared to the controls at P5, 7 and 9 (q = 19.27, p < 0.001; q = 10.45, p < 0.001 and q = 5.97, p < 0.01, respectively) (Fig.. 1B).

Cliff avoidance

A main effect of treatment was found ($F_{(2,23)}$ = 4.05, p < 0.05), as well as the expected effect of age ($F_{(2,23)}$ = 23.05, p < 0.001). However, the interaction was not significant ($F_{(2,23)}$ = 2.40, p > 0.05). Post hoc comparisons confirmed that mice treated with 500 mg/kg of GBH had slower cliff avoidance time than controls only at P 5 (q = 4.84, p < 0.05) (Fig.. 1C).

Traction test
One-way ANOVA analysis showed a significant difference between treated and control groups ($F_{(2,23)} = 21.38$, $p < 0.001$). Post hoc analysis revealed that mice treated with 250 mg/kg or 500 mg/kg GBH showed shorter fall-down latencies than the controls at P10 (250 mg/kg vs control: $q = 2.41$, $p < 0.05$; 500 mg/kg vs control: $q = 6.47$, $p < 0.001$). The difference between the two doses of GBH was also statistically significant (250 mg/kg vs 500 mg/kg: $q = 4.06$, $p < 0.01$) (Fig.. 1D).

Rotarod test
The two-way ANOVA analysis revealed that the motor coordination was significantly affected by the treatment ($F_{(2,23)} = 13.61$, $p < 0.001$), as well as by the age of the animals ($F_{(2,23)} = 64.04$, $p < 0.001$). Likewise, the interaction was significant ($F_{(2,23)} = 4.76$, $p < 0.001$). Multiple comparisons confirmed that both GBH-treated groups had lower fall-down latency than the control group on P23 (250 mg/kg vs control: $q = 4.95$, $p < 0.05$; 500 mg/kg vs control: $q = 8.01$, $p < 0.001$) and P24 (250 mg/kg vs control: $q = 4.83$, $p < 0.05$; 500 mg/kg vs control: $q = 5.42$, $p < 0.01$) (Fig.. 1E).

Effects of GBH on offspring lasting into adulthood
To investigate whether GBH exposure during prenatal and postnatal developmental might translate into long-lasting consequences on adult behaviour, we tested the behavioural repertoire of adults' offspring by assaying locomotor activity, anxiety-like phenotype, social interaction as well as different forms of memory.

Open field test
As indicators of locomotor activity and anxiety-like levels, we recorded the total distance travelled over the open field as well as the velocity and the time spent in the central zone of the maze (anxiety index). One-way ANOVA analysis of the total distance travelled and the percentage of the time spent in the central zone revealed significant differences between treated and control groups ($F_{(2,17)} = 3.76$, $p < 0.05$; $F_{(2,17)} = 50.67$, $p < 0.001$, respectively). However, no difference was found between groups for the velocity ($F_{(2,17)} = 3.76$, $p > 0.05$) (Fig.. 2C), even though treated mice seemed slower than controls. Multiple comparisons confirmed that the group of 500 mg/kg exhibited significant decrease of distance travelled compared to the control group ($t = 2.69$, $p < 0.05$) (Fig.. 2B). Similarly, post hoc analysis revealed that both GBH-treated groups spent significantly less time in the center of the open field compared to the control (250 mg/kg vs control: $t = 6.57$, $p < 0.001$; 500 mg/kg vs control: $t = 9.88$, $p < 0.001$). In addition, the same analysis revealed a significant difference between treated groups ($t = 3.30$, $p < 0.01$) (Fig.. 2D).

Elevated plus-maze
The open field result was confirmed by EPM data. Indeed, one-way ANOVA analysis of the ratio of time spent in the OA and the anxiety index showed a significant difference between treated and control group ($F_{(2,17)} = 72.38$, $p < 0.001$; $F_{(2,17)} = 18.17$, $p < 0.001$, respectively). However, the analysis of the ratio of entries' number in the OA did not show any statistical difference between groups ($F_{(2,17)} = 1.05$, $p > 0.05$) (Fig.. 2G). Multiple comparisons confirmed that the ratio of time spent in the OA was significantly lower in treated groups compared to the control group (250 mg/kg vs control: $t = 9.29$; $p < 0.001$; 500 mg/ kg vs control: $t = 11.26$; $p < 0.001$) (Fig.. 2F). Moreover, the anxiety index was significantly higher in treated groups (250 mg/kg vs control: $t = 4.36$; $p < 0.001$; 500 mg/kg vs control: $t = 5.78$; $p < 0.001$) (Fig.. 2H).

Fig.. 1*: Pre-and postnatal GBH exposure resulted in neurodevelopmental endpoints changes. A) Righting reflex test. B) Negative geotaxis test. C) Cliff avoidance test. D) Traction test. e Rotarod test. Results are presented as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001. The "*" refers to 250 mg/kg or 500 mg/kg vs control groups comparison

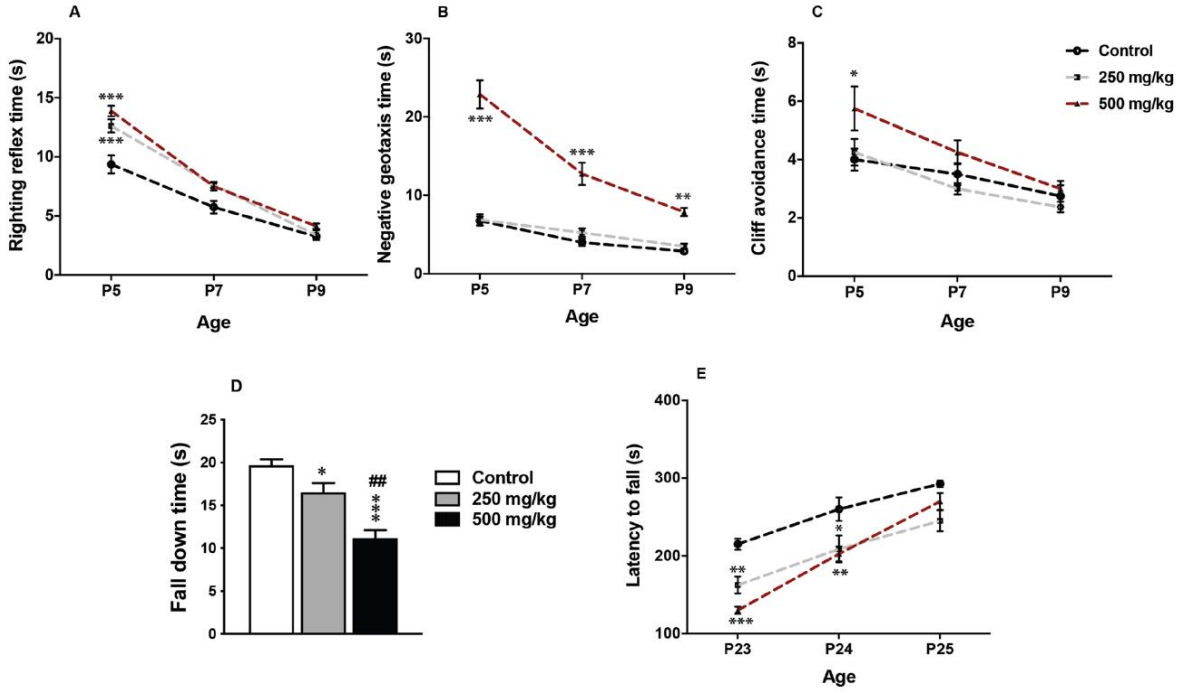


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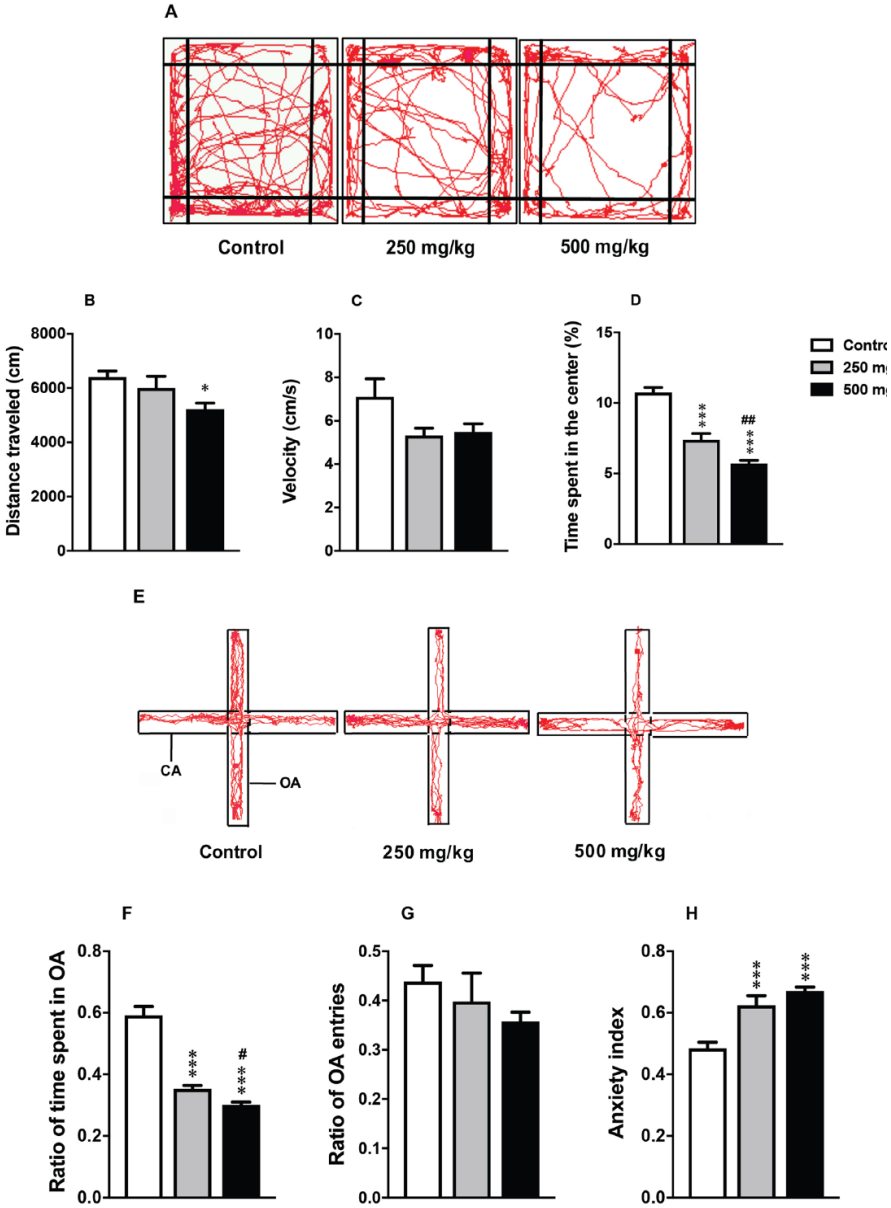
Fig.. 2*: Pre- and postnatal GBH exposure resulted in behavioral alterations in the offspring during adulthood. A) Recording of the trajectory of in the OF test. B–D) Effect of GBH on locomotor activity and anxiety-like phenotype in the open field test. E) Recording of the trajectory of mice in the EPM test. F–H) Effect of GBH on anxiety-like phenotype in EPM test. Results are presented as mean ± SEM. *p < 0.05; ***p < 0.001; #p < 0.05; ##p < 0.01. The "*" refers to 250 mg/kg or 500 mg/kg vs control groups comparison and the "#" refers to the 250 mg/kg vs 500 mg/kg groups comparison. OA open arm, CA closed arm



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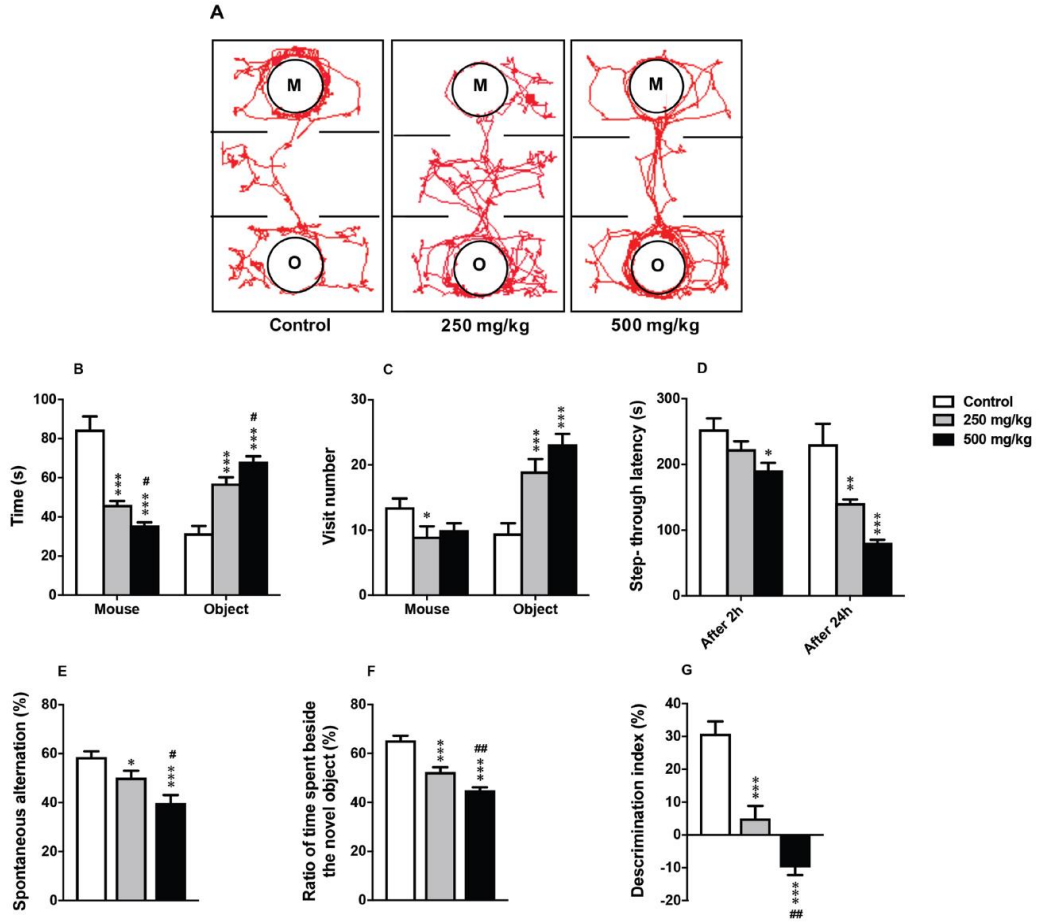
Three-chambered sociability

This test was used to investigate the voluntary social interaction of animals. The data analysis revealed a significant interaction between the wire cup (holding mouse vs object) and treatment ($F_{(2,17)}$ = 73.53, p < 0.001). However, there was no significant main effect of both treatment and wire cup $F_{(2,17)}$ = 2.00, p > 0.05; $F_{(2,17)}$ = 1.10, p > 0.05, respectively). Post hoc comparisons revealed that mice treated either with 250 or 500 mg/kg of GBH spent less time with the wire cup holding another conspecific (250 mg/kg vs control: t = 7.42, p < 0.001; 500 mg/kg vs control: t = 9.42, p < 0.001; 250 mg/ kg vs 500 mg/kg: t = 1.99, p < 0.05) and more time with the wire cup holding the object (250 mg/kg vs control: t = 4.89, p < 0.001; 500 mg/kg vs control: t = 7.06, p < 0.001; 250 mg/kg vs 500 mg/kg: t = 2.16, p < 0.05) compared to the controls (Fig.. 3B). We also found a significant effect of the wire cup factor on the visit number as well as its interaction with the treatment factor ($F_{(2,17)}$ = 26.25, p < 0.001; $F_{(2,17)}$ = 17.89, p < 0.001, respectively). Multiple comparisons confirmed that only mice

exposed to 250 mg/kg showed less visit number of wire cup holding another conspecific ($t = 4.89$, $p < 0.001$), while both GBH-exposed groups showed higher visit number of the wire cup holding the object compared to the controls (250 mg/kg vs control: $t = 4.39$, $p < 0.001$; 500 mg/kg vs control: $t = 6.32$, $p < 0.001$) (Fig.. 3C).

Fig.. 3*: Pre- and postnatal GBH exposure resulted in sociability and cognitive alterations in the offspring during adulthood. A) Recording of the trajectory in the TCS test. B) and C) Effect of GBH on social interaction in the TCS test. D) Effect of GBH on short and long-term memory in the PA test. E) Effect of GBH on working memory in the Y-maze test. F) and G) Effect of GBH exposures on recognition memory in the NOR test. Results are presented as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001; #p < 0.05; ##p < 0.01. The "*" refers to 250 mg/kg or 500 mg/kg vs control groups comparison and the "#" refers to the 250 mg/kg vs 500 mg/kg groups comparison. M mouse, O object



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Passive avoidance

We further examined mice by step-through avoidance learning, an experimental paradigm used for assessing learning, short- and long-term memory. The change in the latency to enter into the dark compartment was compared among the three treatment groups and was found to be affected by both treatment and time of test ($F_{(2,17)} = 13.96$, $p < 0.01$; $F_{(2,17)} = 31.00$, $p < 0.01$, respectively). The analysis revealed also a significant interaction between the two factors ($F_{(2,17)} = 7.46$, $p < 0.05$). Post hoc analysis indicated that only the group exposed to 500 mg/kg of GBH showed a significant decrease of latency after 2 h (500 mg/kg vs control: $t = 6.32$, $p < 0.001$), while both treated groups exhibited a significant decrease of latency 24 h of after the electrical shock administration (250 mg/kg vs control: $t = 4.39$, $p < 0.001$; 500 mg/kg vs control: $t = 6.32$, $p < 0.001$) (Fig.. 3D).

Y-maze

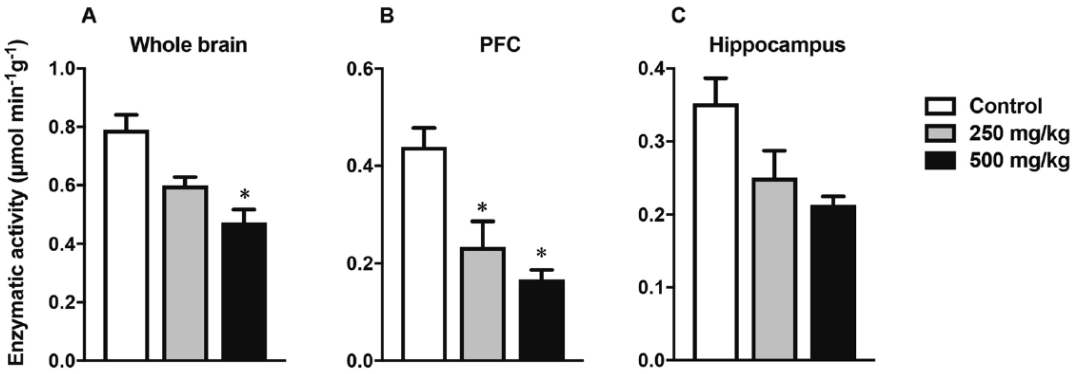
The effect of GBH on working memory was evaluated by Y-maze task. One-way ANOVA analysis showed a significant difference in spontaneous activity between treated and control groups ($F_{(2,17)} = 11.50$, $p < 0.001$). Post hoc comparisons confirmed that the spontaneous alternation in GBH-treated mice was lower than that in control mice (250 mg/kg vs control: $t = 2.14$, $p < 0.05$; 500 mg/kg vs control: $t = 4.78$, $p < 0.001$) and this effect was significantly more pronounced in 500 mg/kg than in 250 mg/kg ($t = 2.64$, $p < 0.001$) (Fig.. 3E).

Novel object recognition

This test was used to assess the potential effects of GBH on recognition memory. One-way ANOVA analysis showed a significant difference in both the ratio of time spent beside the new object and the discrimination index ($F_{(2,17)} = 37.70$, $p < 0.001$). Multiple comparisons revealed that GBH exposed mice spent less time exploring the novel object and had low discrimination index compared to the controls (250 mg/kg vs control: $t = 5.43$, $p < 0.001$; 500 mg/kg vs control: $t = 8.58$, $p < 0.001$). In addition, the same analysis revealed a significant difference between treated groups ($t = 3.15$, $p < 0.01$) (Fig.. 3F and G).

Biochemical, histological and molecular changes within brain of GBH-exposed mice
GBH effects on AChE activity
Cholinergic system is closely associated with anxious and cognitive functions. The impact of GBH exposure on AChE in the supernatants of specific brain area homogenates were assessed. ANOVA analysis showed significant differences between groups in PFC and whole brain ($F_{(2,2)} = 12.78$; $p < 0.05$; $F_{(2,2)} = 13.93$; $p < 0.05$, respectively), while no statistical difference was found in the hippocampus ($F_{(2,2)} = 5.79$; $p > 0.05$) (Fig.. 4C). Post hoc comparisons revealed that only the group exposed to 500 mg/kg of GBH showed a significant decrease of AChE activity in the whole brain (500 mg/kg vs control: $t = 5.24$, $p < 0.05$) (Fig.. 4A), while the activity of this enzyme was significantly decreased in PFC for both doses 250 and 500 mg/kg doses (250 mg/kg vs control: $t = 4.85$, $p < 0.05$; 500 mg/kg vs control: $t = 3.65$, $p < 0.05$) (Fig..4b).

Fig.. 4*: Pre- and postnatal GBH exposure resulted in AChE inhibition. A) Whole brain. B) PFC. C) Hippocampus. Results are presented as mean ± SEM. *p < 0.05. The "*" refers to 250 mg/kg or 500 mg/kg vs control group comparison



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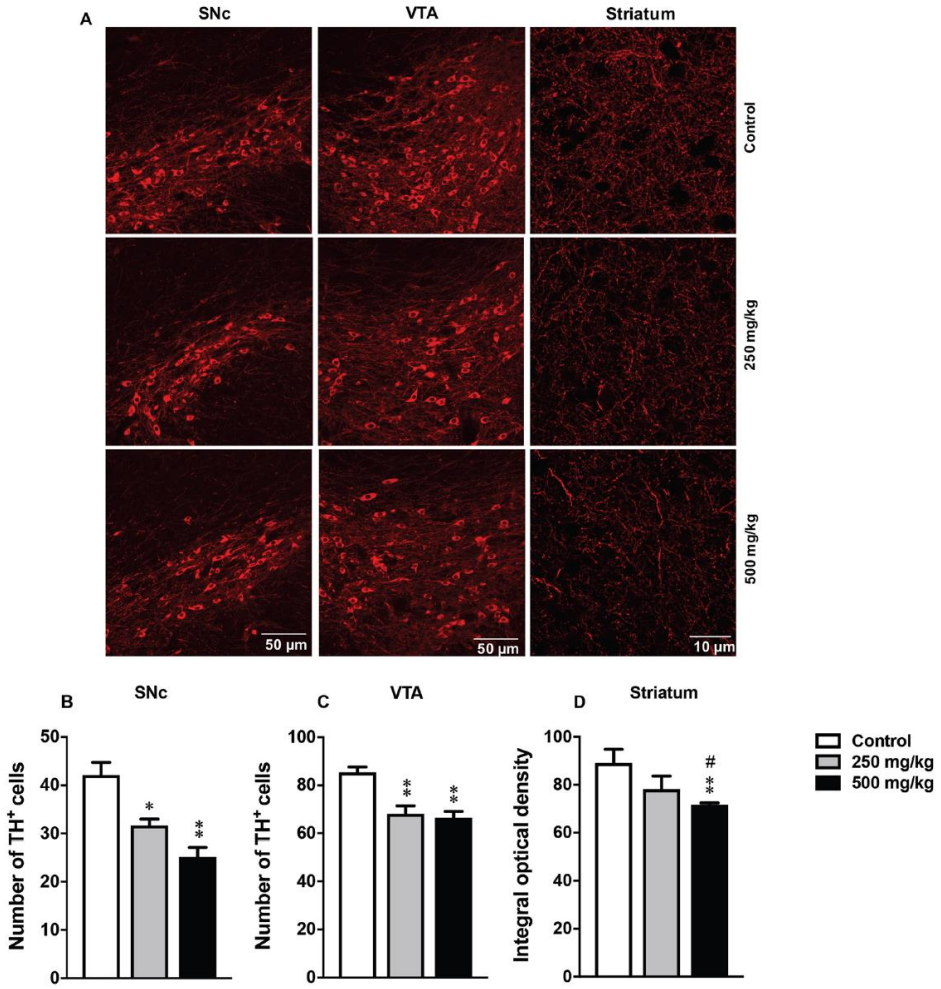
GBH effect on dopaminergic system
To examine the impact of GBH exposure on dopaminergic neurons, we evaluated the expression of tyrosine hydroxylase (TH), a key enzyme involved in dopamine synthesis. Indeed, the quantitative analysis of TH-immunolabeled cells number in the SNc, in the VTA and in the striatum indicated a significant difference between control and treated groups ($F_{(2,17)} = 17.89$, $p < 0.001$; $F_{(2,17)} = 14.78$, $p < 0.01$; $F_{(2,17)} = 8.41$, $p < 0.05$, respectively). Post hoc comparisons confirmed a significant reduction in the number of TH-positive cell bodies (TH+) in the SNc (250 mg/kg vs control: 3.66, $p < 0.05$; 500 mg/kg vs control: $t = 5.92$, $p < 0.01$) (Fig.. 5B) and in the VTA (250 mg/kg vs control: 4.47, $p < 0.01$; 500 mg/kg vs control: $t = 4.91$, $p < 0.01$) of GBH-exposed animals compared to the controls (Fig.. 5C). Moreover, the same analysis showed that 500 mg/kg treated group showed a significant decrease in the integral optical density of the TH+ fibers immunofluorescence in the striatum compared to the control and 250 mg/kg groups (500 mg/kg vs control: $t = 4.07$, $p < 0.01$; 250 mg/kg vs 500 mg/kg: $t = 2.46$, $p < 0.05$) (Fig.. 5D).

Fig.. 5*: Pre- and postnatal GBH exposure resulted in dopaminergic circuit defects. A) Photomicrographs of mice brain cross sections showing the tyrosine hydroxylase (TH)-immunoreactive neurons. B) Count of TH positive cells in the SNc and C) in the VTA. D) The density of TH Immunoreactivity in the striatum. Results are presented as mean ± SEM. *p < 0.05; **p < 0.01; #p < 0.05. The "*" refers to 250 mg/kg or 500 mg/kg vs control groups comparison and the "#" refers to the 250 mg/kg vs 500 mg/kg groups comparison. SNc substantia nigra pars compacta, VTA ventral tegmental area



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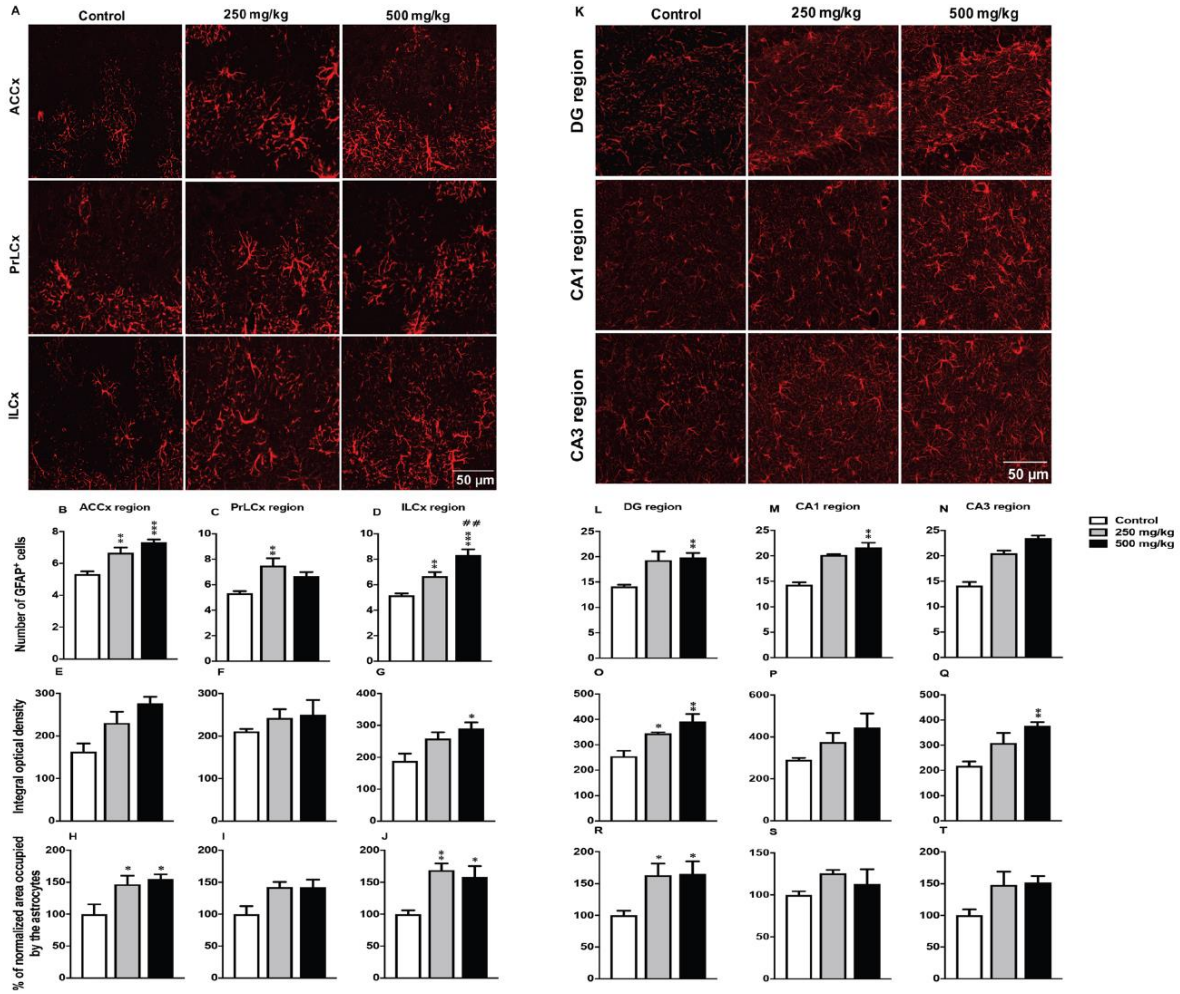
GBH causes neuroinflammation

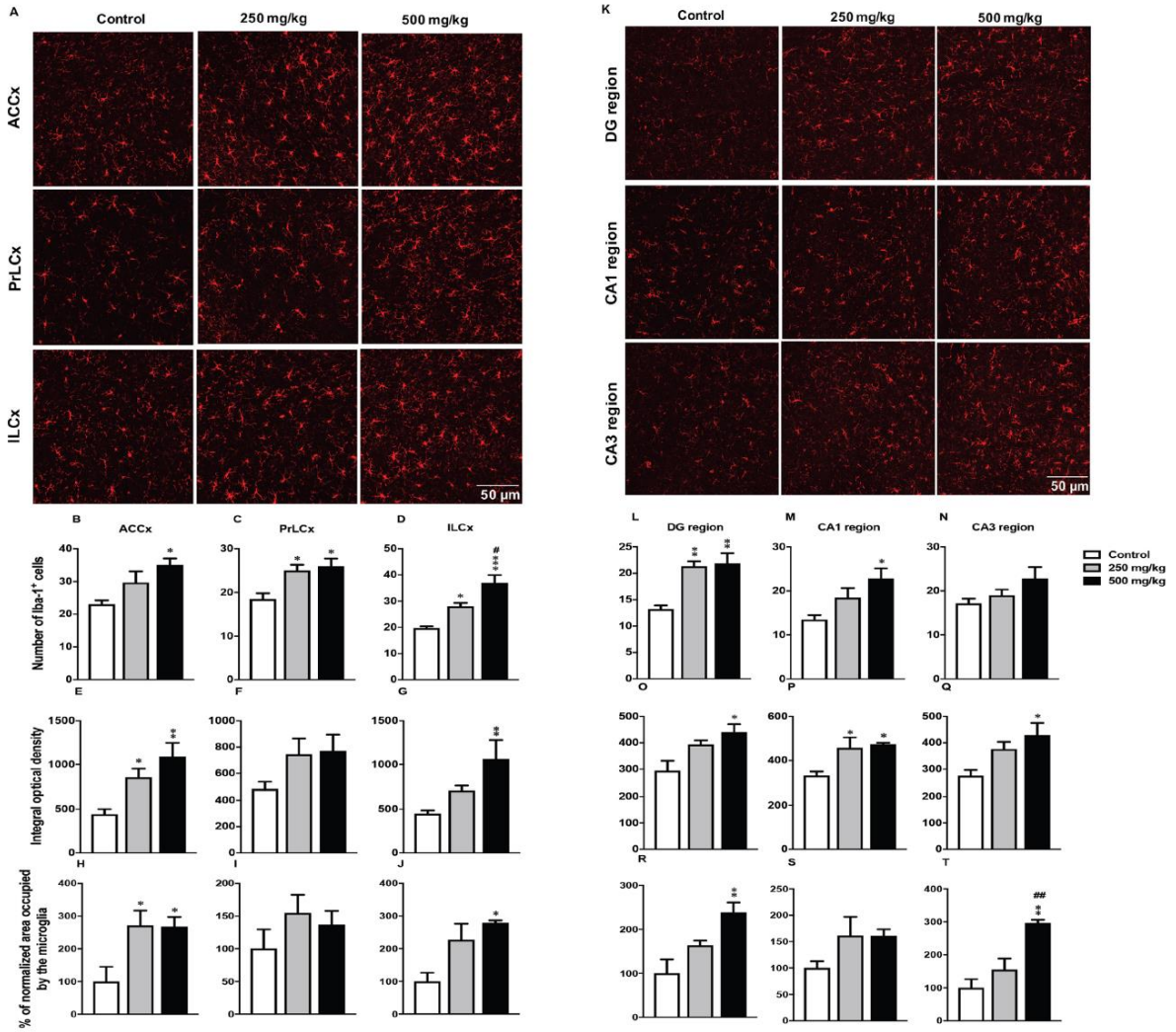
Neuroinflammation was evaluated by assessing the expression of both GFAP and Iba-1 proteins in astrocytes and microglial cells, respectively, in different regions of the PFC and the dorsal hippocampus. Interestingly, the quantitative analysis of GFAP and Iba-1 immunofluorescence showed a significant difference among groups. The treated groups showed a significant increase in the number, integral optical density and area occupied by GFAP+ and Iba-1+ cells in both PFC and hippocampus (Fig.. 6 and 7).

Fig.. 6*: Pre- and postnatal GBH exposure resulted in reactive astrocytes. A) and K) Micrographs showing the expression of GFAP by immunofluorescence in the PFC and hippocampus. B)–D) Count of GFAP positive cells in the PFC and L)–N) in the hippocampus. E)–G) The integral optical density of GFAP positive cells in the PFC and O)–Q) in the hippocampus. H)–J) Area occupied by GFAP positive cells in the PFC and R)–T) in the hippocampus. Results are presented as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001; ##p < 0.01. The "*" refers to 250 mg/kg or 500 mg/kg vs control groups comparison and the "#" refers to the 250 mg/kg vs 500 mg/kg groups comparison. ACCx anterior cingulate cortex, PrLCx prelimbic cortex, ILCx infralimbic cortex, DG dentate gyrus, CA1 Ammon's horn 1, CA3 Ammon's horn 3



*Retrived from Ait-Bali, Y., *et al*., Archives of Toxicology (2020) 94: 1703-1723

Fig.. 7*: Pre- and postnatal GBH exposure resulted in reactive microglia. A) and K) Micrographs showing the expression of Iba-1 by immunofluorescence in the PFC and hippocampus. B)–D) Count of Iba-1 positive cells in the PFC and L)–N) in the hippocampus. E)–G) The integral optical density of Iba-1 positive cells in the PFC and O)–Q) in the hippocampus. H)–J) Area occupied by Iba-1 positive cells in the PFC and R)–T) in the hippocampus. Results are presented as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001; #p < 0.05; ##p < 0.01. The "*" refers to 250 mg/kg or 500 mg/kg vs control groups comparison and the "#" refers to the 250 mg/kg vs 500 mg/kg groups comparison. ACCx anterior cingulate cortex, PrLCx prelimbic cortex, ILCx infralimbic cortex, DG dentate gyrus, CA1 Ammon's horn 1, CA3 Ammon's horn 3



*Retrived from Ait-Bali, Y., *et al*., Archives of Toxicology (2020) 94: 1703-1723

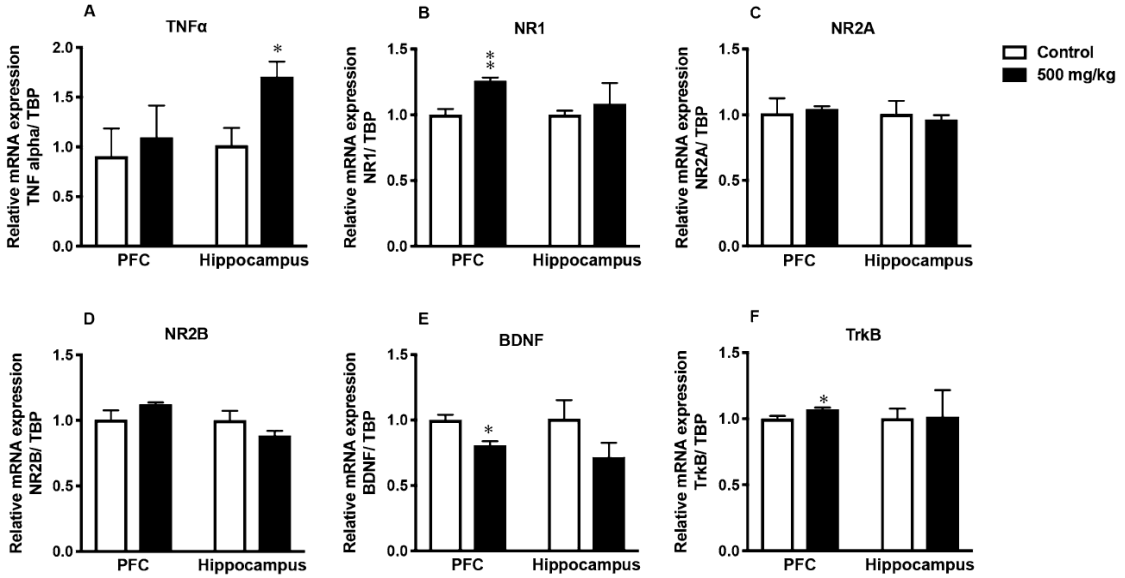
GBH affects the expression of genes associated with neuroinflammation, synaptic plasticity and cell survival

One major hallmark of the inflammatory response is the release of cytokines and chemokines from activated glial cells, we measured the mRNA expression of tumor necrosis factor alpha (TNFα) by quantitative real-time PCR (qRTPCR). The statistical analysis showed significant increased levels of TNFα mRNA expression in the hippocampus of exposed mice compared to controls ($t = 5.26$, $p < 0.05$), while no statistical difference was found in the PFC ($t = 0.41$, $p > 0.05$) (Fig.. 8A). To test whether GBH could impact glutamatergic signaling, the expression level of genes encoding for different NMDA receptors subunits were assessed. Our results showed that GBH induced significant increase in the mRNA expression of NR1 subunit in the PFC ($t = 4.92$, $p < 0.01$) (Fig.. 8B), while no significant differences could be observed for both NR2A and NR2B subunits in either the PFC or the hippocampus ($p > 0.05$) (Fig.. 8C and D).

Based on data related to cognitive deficits, the impact of GBH exposure on the expression of brain derived neurotrophic factor (BDNF) and its receptor tyrosine regulated kinase B (TrkB) within the PFC and hippocampus was assessed. The analysis showed a statistically significant decreasing effect of GBH on the expression of BDNF ($t = 3.76$, $p < 0.05$) and a significant increasing effect on the expression of its receptor TrkB

(t = 3.08, p < 0.05) in the cortices of treated mice (Fig.. 8E and F). However, no statistical significant differences were found in the hippocampi (t = 1.65, p > 0.05; t = 0.05, p > 0.05, respectively) (Fig.. 98E and F).

Fig.. 8*: Pre- and postnatal GBH exposure resulted in genes expression in the PFC and hippocampus of adult progeny. A) qRT-PCR analysis of TNFα transcript. B)–D) qRT-PCR analysis of NMDA receptor subunits transcripts. E) qRT-PCR analysis of BDNF and F) its receptor TrkB transcripts. For each tissue (PFC, Hippocampus), the gene expression is shown relatively to its control samples. Results are presented as mean ± SEM. *p < 0.05; **p < 0.01. The "*" refers to 250 mg/kg or 500 mg/kg vs control groups comparison



*Retrived from Ait-Bali, Y., *et al*., Archives of Toxicology (2020) 94: 1703-1723

Conclusion (by the author)

The results indicate that GBH exposure to female mice of 250 and 500 mg/kg during pregnancy and lactation induces multiple behavioral abnormalities involving motor, emotional, social and cognitive functions and targets CNS integrity, affecting cholinergic, dopaminergic and glutamatergic systems as well as neuroinflammation and cellular stress induction. These results strongly shed light on additional (noncholinergic) mechanisms mediating neurological injury induced by GBH.

Assessment and conclusion

Assessment and conclusion by applicant:

Relevant but supplementary information: *In vivo* study on pre and post natal effects of Roundup on swiss mice at 2 different doses only, no OECD guideline followed, no GLP status stated, no HCD provided. Oral gavage dosing of formulated product is not relevant to real life exposure scenarios. Environmental fate and metabolism for glyphosate active ingredient versus surfactants are different, and oral co-exposures to mammals at the excessively high doses tested in this case are considered irrelevant to human health risk assessment. In addition, insufficient information is provided to determine which formulation was tested and whether it is the glyphosate EU representative formulation.

Assessment and conclusion by RMS:

In this study, groups of 10 female Swiss mice received Roundup by gavage at concentrations of 250 or 500 mg/kg bw/day from G0 to PND21. Following PND60 the downstream effects at the behavioural, neurochemical and molecular levels were examined. The results show that pre- and neonatal exposure to GBH impairs fertility and reproduction parameters as well as maternal behaviour of exposed mothers. In offspring, exposed animals show a delay in innate reflexes and a deficit in motor development. At the adult age, exposed animals showed a decrease of locomotor activity, sociability, learning and short- and long-term memory associated with alterations of cholinergic and dopaminergic systems. GBH also activated microglia and astrocytes, sign of neuroinflammation event in the medial prefrontal cortex and hippocampus. At the molecular level, a downregulation of BDNF expression and an up-regulation of TrkB, NR1 subunit of NMDA receptor as well as

TNFα were found.

The study is considered as supplementary data. The study is reliable with restrictions because of the following reasons: formulation used, only two doses tested, no OECD guideline followed, no GLP status stated, no positive control used and no HCD provided.

B.6.8. OTHER TOXICOLOGICAL STUDIES

B.6.8.1. Toxicity studies on metabolites and relevant impurities

B.6.8.1.1. Studies with AMPA

B.6.8.1.1.1. Absorption, Distribution, Metabolism and Excretion

Study 1

Data point:	CA 5.8.1/001
Report author	[redacted]
Report year	1973
Report title	Final Report on CP 67573 RESIDUE AND METABOLISM Part 11: The Metabolism of Aminomethylphosphonic Acid-^{14}C (CP 50435-^{14}C) in the Laboratory Rat
Report No	303
Document No	NA
Guidelines followed in study	None stated
Deviations from current test guideline	Yes, reporting deficiencies (no batch or purity of test material, only one dose, only 5 days)
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, study conducted prior to GLP
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 3a **Conclusion AGG:** The study is considered to be unacceptable as the study report is very brief and results are only limited described. It is noted that the tested dose level is quite low compared to the dose levels tested in the repeated dose toxicity studies.

2. Full summary

Executive summary

These studies were initiated to investigate the metabolic fate of aminomethylphosphonic acid-^{14}C ([^{14}C]-AMPA), in the rat following a single oral dose to rats.

Male Wistar rats received a single dose of approximately 6.7 mg [^{14}C]-AMPA/kg bw by oral gavage and were sacrificed 120 hours (5 days) after dosing. During this period, urine, faeces and expired gases were collected at 12 or 24 hour intervals and assayed for radioactivity. At sacrifice, blood and selected tissues were examined for radioactive residues.

74 % of the applied dose was detected in faeces and 20 % in urine with less than 0.1 % expired as $^{14}CO_2$ 120 h after application of which most was excreted within the first 24 hours. Therefore, the recovery of administered radioactivity in excreta exceeded 90 %. Approximately 0.06 % of the original dose was recovered from the carcass at 120 hours post-administration, but liver, kidney and muscle exhibited residues of only 6, 6 and 3 ppb, respectively.

Chromatographic and spectral data have demonstrated that orally administered [C^{14}]-AMPA is excreted unchanged in the urine.

I. MATERIALS AND METHODS

A. MATERIALS

1. Radiolabelled test material:	Aminomethylphosphonic acid-^{14}C
Identification:	Aminomethylphosphonic acid-^{14}C (CP 50435-^{14}C) (AMPA-^{14}C)
Position of radiolabel:	Not stated
Lot/Batch #:	Not reported
Purity:	Not reported
Specific activity:	8.9 mCi/mmol
Stability of test compound:	Not reported
2. Test animals:	
Species:	Rat
Strain:	Wistar (SPF)
Source:	[redacted]
Age:	Not stated, but about 6 to 8 weeks considering the body weight
Sex:	Male
Weight at dosing:	Approximately 150 g
Acclimation period:	Not reported
Diet/Food:	*ad libitum*, fasted 4 hours prior to dose
Water:	*ad libitum*
Housing:	Individually in Roth metabolism cages
Environmental conditions:	Temperature: not reported Humidity: not reported Air changes: not reported

B. STUDY DESIGN

Animal assignment and treatment

Male rats were fasted for four hours, before application of an aqueous solution containing approximately 1 mg of [^{14}C]-AMPA (corresponding to a dose of 6.7 mg/kg bw) by gavage. Urine, faeces and the 1N NaOH used to trap the expired gases were collected after l2, 24, 48, 72, 96 and 120 hours. At termination a heparinised blood sample was taken by cardiac puncture under light ether anaesthesia. The animal was sacrificed by continued ether anaesthesia and the following tissues removed: liver, kidney, spleen, heart, brain, testes, fat, muscle and the gastrointestinal tract (GIT) from the oesophagus to the rectum including the caecum.

Measurement of radioactivity in excreta and expired gases

One ml of the NaOH trapping solution was diluted with 2 mL water, and 15 mL phosphor solution (Insta-Gel-Packard Instrument Co., Downers Grove, Ill.) added and the sample analysed for $^{14}CO_2$ by liquid scintillation counting.

Urine was analysed for ^{14}C-activity by diluting 0.1 mL and 0.2 mL aliquots with 4 mL 0.1M NH_4HCO_3. 15 mL phosphor solution was added and the mixture shaken vigorously and chilled to form a gel. The samples were then analysed by liquid scintillation counting.

Faeces was homogenised in 30 % aqueous isopropyl alcohol. The faeces homogenate was lyophilized and 100 mg aliquots submitted for combustion. The trapped ^{14}C-activity was analysed by liquid scintillation counting.

Measurement of radioactivity in tissues

The collected tissue samples were weighed, frozen and lyophilized. The lyophilized samples were weighed, aliquoted and submitted for combustion and subsequent liquid scintillation counting.

Isolation of AMPA-^{14}C from rat urine

A cation-exchange column was prepared by pouring aqueous slurry of AG-50W-X8 (H^+ form; 200/400 m). The resin bed was washed with distilled water until the eluate was colourless. A 12 or 24 hour urine sample which had been diluted to 25-35 mL with distilled water was loaded onto this cation exchanger. The column was then eluted with 1500 mL distilled water and 20 mL fractions were collected. The eluent fractions which contained radioactivity were pooled and loaded onto an anion-exchanger of AG-1-x8 (HCO_3^- form, 200/400 m). The resin was prepared from the Cl^- form by passing 1M NH_4 HCO_3 (2L 1M NH_4 HCO3/1b resin) through the resin. The resin was then washed with water until the eluate was neutral. An amount of resin equivalent to 20 g dry weight was slurried with water and poured into a glass column forming a resin bed. The sample was then applied in a volume of 200 mL. The column was eluted with 200 mL distilled water, followed by 300 mL 0.2N NH_4HCO_3. Fractions of 5 or 10 mL were collected.

The eluent fractions from the anion exchanger which contained radioactivity were pooled and reduced to 1 – 1.5 mL and loaded onto a Bio-Gel P-2 column for gel-filtration chromatography. The gel was prepared by suspension in 0.5N acetic acid, deaerator with a water aspirator and pouring the slurry into a column to form a bed of 1 x 108 cm. After the sample was charged onto the column, the sample flask was rinsed with 1 - 2 mL distilled water which was added to the column. The column was then eluted with 200 mL 0.5N acetic acid and 1 - 2 mL fractions collected.

Authentic standards of radiolabelled AMPA added to urine from untreated rats were eluted quantitatively from the cation exchange resin. Those fractions containing ^{14}C-activity were then pooled, reduced in volume and chromatographed on an anion exchanger and subsequently on a gel filtration column. Typically, overall recoveries of ^{14}C-activity from [^{14}C]-AMPA enriched rat urine were approximately 70 % of initial ^{14}C-activity. Urine from rats orally administered [^{14}C]-AMPA which was chromatographed in the same way resulted in approximately 60 % recovery of the initial ^{14}C-activity present in the crude urine.

Thin layer chromatography (TLC)

The Bio-Gel fractions which contained radioactivity were pooled reduced to small volume and an aliquot applied to a 20 x 20 cm thin-layer chromatography (TLC) plate with a 250 µ layer of microcrystalline cellulose. The plates were developed first in a phenol-water system of the following composition:

90 % Phenol (Fisher Certified)	84 mL
Distilled Water	16 mL
Glacial Acetic Acid	1 mL
EDTA	37.2 mg

After the plates were air-dried, they were rotated 90° and developed in a modified semi-stench solvent system of the following composition:

EDTA	1.2 g
17N NH_4OH	100 mL
Distilled Water	475 mL
l-Propanol	350 mL
2-Propanol	75 mL
l-Butanol	75 mL
Iso-Butyric Acid	2500 mL

Colorimetric visualization of the TLC plates was accomplished by spraying with or with a modified Hanes reagent. ^{14}C-activity on the TLC plates was detected by means of the Beta Camera, Model 6000. A permanent copy of the Beta Camera CRT image was reproduced using a Polaroid Pack Camera.

Nuclear Magnetic Resonance (^{1}H-NMR) of the isolated fractions

The samples were lyophilized and exchanged twice with 99.8 % deuterium oxide (D_2O). The sample was then dissolved in 0.1 mL 100 % D_2O. Immediately before running the spectra, the samples were dissolved in 10 µl 100 % D_2O and the solution filtered through a Flath-Ludin syringe filter directly into a capillary tube. An additional 5 µL D_2O was used to rinse the vial and was also filtered directly into the capillary tube. The capillary was inserted into a teflon chuck which was then placed in a 7 inch NMR tube (Wilmad, No. 529-PP).

High resolution proton spectra (60 mHz) were run on a Varian T-60 and/or a JEOL JNM C-60-HL spectrometer. The latter was equipped with a JNM-AS-1 resolution stabilizer, JRA-1 spectrum accumulator, Monsanto 100-A frequency counter, an external Hewlett Packard Model 200 CD wide range audio oscillator, a Hewlett Packard Model 5245L electronic counter, a hetero spin decoupler (JNM-5D-HC) and an RF oscillator adapter (JNM-OA-1). All ^{1}H-NMR spectra were calibrated using HOD as the internal reference and were decoupled using the hetero spin decoupler (JNM-5D-HC) and an RF oscillator adapter (JNM-OA-1).

Gas-Liquid Chromatography (GLC) of the isolated fractions

Following NMR analysis the samples were derivatized using trifluoroacetic acid, trifluoroacetic anhydride, diazobutane and acetic acid in benzene and examined by GLC on a Perkin-Elmer Model 900 which is a dual column instrument equipped with thermal conductivity (TC), flame ionization (FID) and phosphorus specific (FPD) detectors. The samples were analysed on a glass column packed with 1 – 5 % OV-17 on Chromosorb W-HP (80/100 m) programed from l20 – 240 °C at 10 °C/min.

Mass Spectrometry of the isolated fractions

The final analysis of the purified fractions was performed by coupled gas chromatography and mass spectrometry. A PE-900 GLC was coupled through a Rieman separator to a Perkin-Elmer Model 270 mass spectrometer (MS) operating at 70 ev in the GLC mode. A Honeywell 2106 Visicorder was employed to focus the instrument and record the mass spectra. The PE-270 MS was interfaced through a Hall probe to a Varian SS-100MS data system.

II. RESULTS AND DISCUSSION

A. DISTRIBUTION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF [^{14}C]-AMPA

The major route of excretion of orally administered [^{14}C]-AMPA is via the faeces. More than 50 % of the administered dose was excreted in the faeces within 24 hours and a total of 74 % within 120 hours.

13 % of the dose was recovered from the urine within 12 hours after administration, 4.5 % was detected urine collected 12 - 24 hours after application and less than 3 % was detected in urine collected from 24 - 120 hours. Thus, most of the absorbed material was excreted within 24 hours.

Very little of the absorbed material was catabolised. Less than 0.1 % of the administered ^{14}C-activity was recovered in expired air.

Table B6.8.1.1.1.1-1: Distribution of ^{14}C-activity in male rats orally administered [^{14}C]-AMPA

Time (Hour)	Percent dose recovered[1]					
	Urine	**Faeces**	**CO_2**	**Washes[2]**	**Tissues**	**Cumulative**
12	13.04	5.86	0.06			18.95
24	4.50	47.24	0.01			70.70
48	1.52	19.30	0.00			91.52
72	0.66	0.91	0.00			93.09
96	0.23	0.12	0.00			93.44
120	0.05	0.06	0.00			93.55
Total	**20.00**	**73.49**	**0.06**	**0.13**	**0.06**	**93.74**

[1]Dose was 6.7 mg/kg body weight

[2]Consists of 0.1M NH_4HCO_3 washes of cage and urine-faeces separator

Approximately 0.06% of the administered ^{14}C-activity could be accounted for in the carcass 120 hours following

a single oral dose. Of that ^{14}C-activity the muscle, due to its large relative mass, i.e., 3 % of the total live weight, accounted for approximately one-third of the ^{14}C-activity. However, the concentration calculated on the basis of AMPA-^{14}C equivalents was only 3 ppb in fresh tissue. In addition, the liver and kidney were only 6 ppb.

Table B6.8.1.1.1.1-2: Radioactivity in tissues 120 h after single oral dose of 6.7 mg [^{14}C]-AMPA/kg bw (in µg/g fresh tissue)

Tissue	% Dose	[^{14}C]-AMPA equivalents (µg/g Fresh tissue)
Liver	0.01	0.006
Kidney	<0.01	0.006
Muscle	0.02	0.003
Fat	0.01	0.004
Gut	0.02	0.008
Spleen	<0.01	0.004
Heart	<0.01	0.004
Brain	<0.01	0.001
Testes	<0.01	0.003
Blood	<0.01	0.003

B. CHARACTERIZATION OF THE EXCRETED RADIOACTIVITY.

The purification and spectral methods employed were defined on a urine sample from an untreated control rat. The urine was spiked with approximately 500 µg [^{14}C]-AMPA. The TLC screening procedure is capable of separating the following compounds: glyphosate, AMPA, N-methylaminomethylphosphonic acid, glycine, sarcosine, methylphosphonic acid and hydroxymethylphosphonic acid.

Recovery of ^{14}C-activity from [^{14}C]-AMPA enriched rat urine was approximately 70 % of initial ^{14}C-activity. Urine from rats orally administered [^{14}C]-AMPA which was chromatographed in the same way resulted in approximately 60 % recovery of the initial ^{14}C-activity present in the crude urine.

The purified urine samples were analysed by ^{1}H-NMR after being exchanged with D_2O. The sample isolated from the urine of rats administered [^{14}C]-AMPA exhibited the characteristic doublet due to the methylene protons, although it was not as well defined as in the sample enriched with [^{14}C]-AMPA. However, the chemical shifts for the methylene protons are consistent with those determined for authentic standards, and the ^{31}P-decoupled spectrum shows a clear enhancement in the intensity of the response.

The samples which had been analysed by ^{1}H-NMR were exchanged with water and derivatised for GLC/MS analysis. The mass spectrum of the Di-n-butyl-*N*-trifluoromethyl derivative of the sample isolated from urine enriched with [^{14}C]-AMPA yielded the same basic fragmentation pattern as the spectrum of the standard AMPA-Di-n-butyl-TFA.

Beside the peak of AMPA-Di-n-butyl-TFA additional peaks were observed in urine (P+2) spiked with [^{14}C]-AMPA contributing significantly to fragments containing the ^{14}C-enriched methylene group. In the sample in which [^{14}C]-AMPA was added to and isolated from an untreated urine sample, there are four fragments showing a significant P+2 peak.

The mass spectra of the sample isolated from rat urine have the same characteristic fragmentation pattern as the standard AMPA. The relative intensities of some of the lower mass fragments vary from the standard, but these are not as significant in identification as the higher mass fragments which agree well with the standard.

III. CONCLUSIONS

Orally administered AMPA is only moderately absorbed from the gut of the rat after single application of 6.7 mg/kg bw. Consequently, the faeces represent the major route of excretion of ingested AMPA. Approximately 20 % of the material is absorbed and most of that amount is rapidly excreted unchanged in the urine. Of the material absorbed, very small amounts are catabolized as evidenced by the facts that less than 0.1 % of the dose

is expired in air and tissue residues are less than 10 ppb.

The absorption of [^{14}C]-AMPA from the gut appears to be slightly more rapid than glyphosate since the log unexcreted dose (i.e., body load) versus time plot of AMPA shows only a small distributive phase. However, the biological half-life of [^{14}C]-AMPA of 10-11 hours is of approximately the same magnitude as that of glyphosate which was 8-9 hours. But unlike glyphosate, [^{14}C]-AMPA did not show a redistribution phase at 48-96 hours which plateaued the excretion curve followed by resumption of first-order elimination. Rather at approximately 72 hours the elimination curve simply plateaued as if approaching an asymptote.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The excreta from male rats orally administered [^{14}C]-AMPA were collected at various intervals through 120 hours post-administration and analysed for ^{14}C-activity.

It was found that a total of approximately 74 % of the dose appeared in the faeces and ca 20 % in the urine with less than 0.1 % expired in air. These figures suggest only limited absorption from the gastrointestinal tract. Elimination is rapid and nearly complete. More than 50 % was excreted in the
faeces during the first 24 hours following dosing. About 13 % of the administered dose was found in the urine within the first 12 hours already. Approximately 0.06 % of the total dose only was recovered from the carcass at 120 hours post dose. Liver, kidney and muscle exhibited residues of 6, 6 and 3 ppb, respectively. Chromatographic and spectral data demonstrated that the orally administered AMPA was excreted unchanged in the urine. There was no indication of further metabolism.

The report does not contain any information on the limits of detection or limits of quantitation.

The reporting in this study is very brief and can be considered to be poor and therefore, it is not acceptable based on current regulatory standards.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. Due to the severe reporting deficiencies and limitations of the study it is not considered acceptable. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.8.1.1.2. Acute oral toxicity

Study 1

1. Information on the study

Data point	CA 5.8.1/002
Report author	[redacted]
Report year	1996
Report title	AMPA: Acute Oral Toxicity Study in Mice
Report No	[redacted] 96-0075
Document No	NA
Guidelines followed in study	OECD 401 (1987); US EPA FIFRA Guidelines Subdivision F (1984); Japan MAFF Guidelines 59 NohSan No. 4200 (1985)
Deviations from current test guideline	The study was conducted according to OECD 401 however the deviation was identified according to OECD 420 adopted in 2001 to replace OECD 401. - Clinical signs of toxicity were measured 1 hour after initial dosing, not 30 minutes after initial dosing as recommended by OECD 420.
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 deviations are not expected to significantly impact the outcome of the study.

2. Full summary

Executive Summary
AMPA was evaluated for its acute oral toxicity potential in male and female mice when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the 14-day observation period and no clinical signs were observed at necropsy following the 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, male and female mouse > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	AMPA
Identification:	AMPA
Description:	White powder
Lot/Batch #:	A-960719
Purity:	99.33 %
Stability of test compound:	Stable for 1 year at room temperature
2. Vehicle and/ or positive control:	1 % carboxymethylcellulose
3. Test animals:	
Species:	ICR mice
Strain / Stock:	Crj:CD-1
Source:	[redacted]
Age:	5 weeks (6 weeks after acclimatisation)
Sex:	Male and female
Weight at dosing:	30.5 – 34.6 g for males; 22.9 – 24.8 g for females
Acclimation period:	7 days
Diet/Food:	Certified pellet diet MF (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Groups of 5 animals/sex/cage were housed in aluminium cages with wire-mesh floors
Environmental conditions:	Temperature: 22 ± 3°C Rel. humidity: 55 ± 15 % 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 17 September 1996 to 8 October 1996

Animal assignment and treatment
Based on the results of a preliminary dose-range finding study, 5000 mg/kg bw/day was selected as the dose level for this study.

Table B.6.8.1.1.2.1-1: AMPA: Acute Oral Toxicity Study in Mice ([redacted], 1996): animal distribution

	Dose (mg/kg bw/day)	**Number of Animals Per Group**	
		Males	Females
Test Group	5000	5	5

Animals were fasted for about 3 hours before and 3 hours after administration. The dosing volume was 20 mL/kg bw. Observations for clinical signs of toxicity were made 1, 3, and 6 hours after administration and then once daily thereafter until termination of the observation period. Individual body weights were recorded just prior to dosing, as well as 7 and 14 days after administration. On Day 14 after dosing, all animals were sacrificed under ether anaesthesia and subjected to 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.

D. NECROPSY

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

III. CONCLUSIONS

The oral LD_{50} of the test material (AMPA) in mice was estimated to be greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In a GLP study conducted in accordance with OECD 401 guidelines (1987), the acute oral LD_{50} for AMPA was determined to be greater than 5000 mg/kg bw in male and female mice. The study is considered acceptable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:
It is agreed with the applicant. The acute oral LD_{50} of AMPA in male and female mice was determined to be > 5000 mg/kg bw in this study. This conclusion is in line with the previous assessment (RAR, 2015).

Study 2

1. Information on the study

Data point	CA 5.8.1/003
Report author	[redacted]
Report year	1993
Report title	AMPA: Acute oral toxicity (limit) test in rats
Report No	8763
Document No	NA
Guidelines followed in study	US EPA Pesticide Assessment Guideline Subdivision F, 81-1, FIFRA, OECD
Deviations from current test guideline (OECD 401, 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. It is noted that no clear specification of the OECD guideline is given in the study report, however, the study designed as limit test was conducted in accordance with OECD guideline 401; therefore the study is accepted.

2. Full summary

Executive Summary
The acute oral toxicity of glyphosate's metabolite AMPA was investigated in male and female rats (5 animals/sex/group) of the Sprague-Dawley strain. The test substance, suspended in 0.5 % carboxymethylcellulose, was administered by oral gavage to each animal at a dosage of 5000 mg/kg bw and a 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, diarrhea, subdued behavior, hunched appearance, and soiled anal and perigenital areas. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted.
The acute oral LD_{50} was calculated to be: LD_{50}, oral, female and male rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate metabolite
Identification:	AMPA
Description:	White powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
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:	131 – 175 g
Acclimation period:	7 days
Diet/Food:	Rat and Mouse No. 1 Maintenance diet, *ad libitum* (except the night prior to dosing and 4 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	5 animals/sex/cage in Polypropylene cages with mesh floors suspended over absorbent paper lined trays
Environmental conditions:	Temperature: 18 °C - 21 °C Humidity: 39 % (Average) Air changes: 15 – 20 times per hour 12-hour light/dark cycle (light during 07:00 – 19:00)

B. STUDY DESIGN AND METHODS

In life dates: 01/04/1992 to 15/04/1992 (experimental phase)
Finalisation date: 28/01/1993

Animal assignment and treatment:
Rats were housed by sex (5 males and 5 females) and starved the night prior to dosing. The 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. After dosing, the animals were starved for a further 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 for a period of 14 days. Body weights were recorded prior to administration, day seven and prior to sacrifice on day 14. At study termination all animals were sacrificed by carbon dioxide asphyxiation followed by a gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the 14-days observation period after administration. The oral LD_{50} is above 5000 mg/kg bw for male and female rats.

B. CLINICAL OBSERVATIONS

Clinical signs observed were piloerection, diarrhoea, subdued behaviour, hunched appearance, and soiled anal and perigenital areas. These observations appeared at 4 hours post dosing the first time. Females recovered after day 1, while piloerection in males lasted until day 4 of the study. All clinical signs are listed in table below.

Table B.6.8.1.1.2.2-1: AMPA: Acute oral toxicity (limit) test in rats ([redacted], 1993): Clinical observations

Dose group		**5000 mg/kg bw**									
Sex		**Males**						**Females**			
Time after treatment		**1 min – 2 h**	**4 h**	**1 d**	**2 d**	**3 d**	**4 – 14 d**	**1 min – 2 h**	**4 h**	**1 d**	**2 – 14 d**
Total animals examined	**n**	5	5	5	5	5	5	5	5	5	5
Clinical sign	**n**	0	5	5	3	2	0	0	5	5	0
Piloerection				2	2	2			5	5	
Diarrhea			5	5					5		
Hunched appearance				1	1					3	
Soiled anal and perigenital areas				3						3	
Subdued behaviour									5		

C. BODY WEIGHT

Weight gain of all animals was within the normal range.

D. NECROPSY

No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The oral LD_{50} of the test material (AMPA) in rats was estimated to be greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
As no mortality occurred during the study period, the oral LD_{50} of glyphosate metabolite AMPA in rats is greater than 5000 mg/kg bw. No deviations from the current test guideline were identified, therefore the study is considered acceptable.

Assessment and conclusion by RMS:
It is agreed with the applicant. The acute oral LD_{50} of AMPA in male and female rats was determined to be > 5000 mg/kg bw in this study. All animals showed clinical signs in the course of the study (Table 6.8.1.2.2-1). This conclusion is in line with the previous assessment (RAR, 2015).

Study 3

1. Information on the study

Data point	CA 5.8.1/004
Report author	[redacted]
Report year	1991

Report title	Assessment of acute oral toxicity of (N-methyl-N-phosphonomethyl)glycine to rats
Report No	12837
Document No	NA
Guidelines followed in study	US EPA, subdivision F, Serie 81-1 (1984)
Deviations from current test guideline (OECD 401, 2001)	Control group included in the study. Acclimatization period of 2 days 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 to be acceptable. It is not explicitly stated in the study report that the study was conducted according to OECD guidelines, however, the study designed as limit test was conducted in accordance with OECD guideline 401 (with the deviations noted above); therefore the study is accepted. The deviations noted above are not considered to significantly impact the study outcome.

2. Full summary

Executive summary

The acute oral toxicity of glyphosate's metabolite N-methyl-N-phosphonomethyl)glycine was investigated in male and female rats (5 animals/sex/group) of the Wistar strain. The test substance, suspended in 1 % carboxymethylcellulose, was administered by oral gavage to each animal at a dosage of 5000 mg/kg bw and a constant dose volume of 20 mL/kg bw. The vehicle only was administered to rats of the control group at a dose volume of 20 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, diarrhea, and pinched abdomen. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted. The acute oral LD_{50} was calculated to be: LD_{50}, oral, female and male rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate metabolite
Identification:	(N-methyl-N-phosphonomethyl)glycine
Description:	White powder
Lot/Batch #:	244-KMA-9.1
Purity:	97.3 %
Stability of test compound:	Not specified
2. Vehicle and/ or positive control:	1 % carboxymethylcellulose (CMC)
3. Test animals:	
Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	6 – 7 weeks
Sex:	Male and female
Weight at dosing:	141 – 155 g
Acclimation period:	2 days

Diet/Food:	Complete rodent diet "Altromin 1314", *ad libitum* (except for 18 hours prior to dosing and 3 hours after dosing)
Water:	Water acidified with hydrochloric acid to pH 2.5, *ad libitum*
Housing:	2-3/sex/cage in macrolone cages Type III (42x26x15 cm) with pinewood sawdust bedding
Environmental conditions:	Temperature: 21 ± 3 °C Humidity: 55 ± 15 % (Average) Air changes: 6 times per hour 12-hour light/dark cycle (light during 06:00 – 18:00)

B. STUDY DESIGN AND METHODS
In life dates: 04/07/1991 to 18/07/1991 (experimental phase).
Finalisation date: 14/10/1991

Animal assignment and treatment
Rats were housed by sex (5 males and 5 females) and fasted 18 hours prior to dosing with the test material. The substance was suspended in 1 % CMC and administered by a single oral gavage at a dose level of 5000 mg/kg bw with an application volume of 20 mL/kg bw. After dosing, the animals were fasted for a further 3 hours. A control group was included in the study. The animals of this group received the vehicle only under the same conditions and dose volume as the test group.
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 for a period of 14 days. Body weights were recorded prior to administration, day seven and prior to sacrifice on day 14. At study termination all animals were sacrificed by carbon dioxide asphyxiation followed by a gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the 14-days observation period after administration. The oral LD_{50} is above 5000 mg/kg bw for male and female rats.

B. CLINICAL OBSERVATIONS

Clinical signs observed in the control group included piloerection and pinched abdomen. The animals of the test group showed the same signs and diarrhea in addition. The observations in the test group had resolved on day 2 while observations in the test group persisted until day 3 of the study. All clinical signs are listed in the following table.

Table B.6.8.1.1.2.3-1: Assessment of acute oral toxicity of (N-methyl-N-phosphonomethyl)glycine to rats ([redacted], 1992): Clinical observations

Dose group (mg/kg bw)		**0**					**5000**										
Time after treatment		**1 h**	**3 h**	**5 h**	**1 d**	**2 – 14 d**	**1 h**	**3 h**	**5 h**	**1 d**	**2 d**	**3 d**	**4 d**	**5 d**	**6 d**	**7 d**	**8 – 14 d**
Total animals examined	**n**	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Clinical sign	**n**	10	10	10	3	0	10	10	10	10	9	0	2	1	0	1	0
Piloerection		10	10	10	3F		10	10	10	10	5M/4F		2F	1F		1F	
Pinched abdomen		1M/2F					10	3M/2F	1M								
Diarrhea											4M						

M = males; F = females

C. BODY WEIGHT

Weight gain of all animals was within the normal range.

D. NECROPSY
No abnormal necropsy findings were noticed.

III. CONCLUSIONS
The oral LD_{50} of the test material (N-methyl-N-phosphonomethyl)glycine in rats was estimated to be greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
As no mortality occurred during the study period, the oral LD_{50} of the test material was estimated to be greater than 5000 mg/kg bw. A control group was used in this study, deviating from the current test guideline. However, this is not interfering with the obtained results and the study is considered acceptable.

Assessment and conclusion by RMS:
It is agreed with the applicant. The acute oral LD_{50} of (N-methyl-N-phosphonomethyl)glycine in male and female rats was determined to be > 5000 mg/kg bw in this study. All animals showed clinical signs in the course of the study (Table 6.8.1.2.3-1). This conclusion is in line with the previous assessment (RAR, 2015).

Study 4

1. Information on the study

Data point	CA 5.8.1/005
Report author	[redacted]
Report year	1988
Report title	Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat
Report No	[redacted]/P/2266
Document No	NA
Guidelines followed in study	No guideline specified in the report
Deviations from current test guideline 401	Rats were fasted overnight for up 24 hours whereas OCED guideline 401 states that rats should be fasted overnight only (i.e. not for 24 hours). This deviation might explain why the observed weight gain was not in the normal 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 to be acceptable. Although no guidelines are specified in the study report, the study designed as limit test was conducted in accordance with OECD guideline 401 (with the deviations noted above); therefore the study is accepted.

2. Full summary

Executive summary
Aminomethyl phosphonic acid was evaluated for its acute oral toxicity potential in male and female rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the 14-day observation period. Slight clinical signs of toxicity were observed following dosing but were resolved by Day 4. All animals exhibited a decrease in body weight due to the pre-dose fast; however, body weights normalized by Day 6. Decreased body weights were again observed on Days 6 and 8 for one male, as well as on Days 8 through 15 for one male and three females. 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, male and female rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethyl phosphonic acid
Identification:	Aminomethyl phosphonic acid
Description:	White solid
Lot/Batch #:	Y06384/001/001
Purity:	100% (assumed)
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	0.5 % (w/v) aqueous polysorbate 80
3. Test animals:	
Species:	Wistar-derived albino rats
Strain / Stock:	Alpk:APfSD
Source:	[redacted]
Age:	8 - 9 weeks
Sex:	Male and female
Weight at dosing:	280 - 312 for males; 204 - 214 g for females
Acclimation period:	Minimum of 6 days
Diet/Food:	Porton Combined Diet, Special Diets Services Ltd, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	A maximum 5 animals/sex/cage were housed in suspended cages with stainless steel mesh floor and back
Environmental conditions:	Temperature: 15 – 24 °C Rel. humidity: 50 ± 10 % Air changes: 20 – 30 air changes per hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: Not reported

Animal assignment and treatment
Based on the results of a preliminary dose-range finding study, 5000 mg/kg bw/day was selected as the dose level for this study.
Animals were fasted overnight for a period of up to 24 hours prior to dosing. One female was accidentally killed on Day 1 and was therefore substituted with another animal that was dosed one day later. The dosing volume was 10 mL/kg bw. Observations for clinical signs of toxicity were made once 30 and 90 minutes after dosing and again between 4 and 6 hours after dosing. Individual body weights were recorded one the day prior to dosing, the day of dosing and on Days 3, 5 or 6, 8, and 15. At the end of the study, all animals were sacrificed via inhalation of halothane BP and examined for gross abnormalities.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
Slight signs of clinical toxicity were observed in all animals; however, all animals recovered by Day 3 or 4.

Table B.6.8.1.1.2.4-1: Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat ([redacted], 1998): clinical signs of toxicity

	From Days 1 through 15	
	Males	**Females**
Diarrhea	2/5	1/6
Signs of diarrhea	4/5	2/6
Chromodacryorrhea	2/5	2/6
Piloerection	1/5	1/6
Stains around nose	3/5	4/6
Signs of urinary incontinence	1/5	5/6
Ungroomed	5/5	4/6
Reduced splay reflex	0/5	2/6
Urinary incontinence	0/5	1/5

C. BODY WEIGHT

All animals exhibited decreased body weights due to the pre-dosing fast; however, all weights were normalized by Day 6. After Day 6, one male lost weight between Days 6 and 8. Three females and one male also lost weight between Days 8 through 15. The reason for these losses is unclear as there were no associated clinical abnormalities, nor were there any abnormalities at post mortem examination.

Table B.6.8.1.1.2.4-2: Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat ([redacted], 1998): body weight (mean ± standard deviation)

	From Days 1 through 15	
	Males	**Females**
Pre-dosing	293.2 ± 12.1	207.7 ± 3.7
Day 1	263.6 ± 12.3	186.8 ± 2.7
Day 3	285.4 ± 9.4	209.6 ± 3.8
Day 5	n/a	217.0 ± 0.0
Day 6	311.0 ± 9.7	223.0 ± 4.0
Day 8	325.0 ± 51.6	248.2 ± 27.4
Day 15	364.0 ± 12.7	245.0 ± 7.1

n/a: not applicable

D. NECROPSY

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

III. CONCLUSIONS

The oral LD_{50} of aminomethyl phosphonic acid in rats was estimated to be greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In a GLP study conducted in a similar to OECD 420, the acute oral LD_{50} for aminomethyl phosphonic acid was determined to be greater than 5000 mg/kg bw in male and female rats. The study is considered acceptable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:
It is agreed with the applicant. The acute oral LD_{50} of aminomethyl phosphonic acid in male and female rats was determined to be > 5000 mg/kg bw in this study. All animals showed clinical signs in the course of the study (Table 6.8.1.2.4-1) and body weight gain was not normal in all animals (Table 6.8.1.2.4-2). This conclusion is in line with the previous assessment (RAR, 2015).

Study 5

1. Information on the study

Data point	CA 5.8.1/006
Report author	[redacted]

Report year	1973
Report title	Toxicological investigation of: CP 50435 – Lot: XHD-16
Report No	Y-73-19
Document No	NA
Guidelines followed in study	None
Deviations from current test guideline	Doses are not in line with OECD guideline. Animals were observed for 7 days only. Weights were not recorded. No purity or stability of the test substance provided. Information on the test species used and housing conditions is lacking.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, conducted prior to GLP
Acceptability/Reliability	**Conclusion GRG:** Supportive (Category 3a) **Conclusion AGG:** The study is not considered acceptable since it was not conducted according to GLP and substantial information is missing in the study report. In addition, several deviations from the OECD guidelines are noted.

2. Full summary

Executive summary

The acute oral toxicity of glyphosate's metabolite CP 50435 was investigated in male and female rats (5 animals/group; 2 or 3 animals per sex per group). The test substance, suspended in corn oil, was administered by a single oral gavage to each animal at a dosage of 5010 mg/kg bw, 6310 mg/kg bw, 7940 mg/kg bw and 10000 mg/kg bw. Mortality and clinical signs were recorded during the subsequent 7 days. All animals were subjected to a gross necropsy at the end of the study.

Clinical signs noticed were reduced appetite, reduced activity, increasing weakness, slight diarrhoea and collapse. Mortality occurred in 1/5 rats at 6310 mg/kg bw, 2/5 rats at 7940 mg/kg bw and 4/5 rats at 10000 mg/kg bw. No abnormal necropsy findings were noted in animals that survived until the end of the study period.

The acute oral LD_{50} was calculated to be: LD_{50}, oral, female and male rat 8300 mg/kg bw (CI: 7300 mg/kg bw – 9460 mg/kg bw).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate metabolite
Identification:	CP 50435
Description:	Not specified
Lot/Batch #:	XHD – 16
Purity:	Not specified
Stability of test compound:	Not specified
2. Vehicle and/ or positive control:	Corn oil
3. Test animals:	
Species:	Rat
Strain:	Not specified
Source:	Not specified
Age:	Not specified
Sex:	Male and female
Weight at dosing:	205 g – 250 g
Acclimation period:	Not specified
Diet/Food:	Not specified

Water:	Not specified
Housing:	Not specified
Environmental conditions:	Temperature: Not specified Humidity: Not specified

B. STUDY DESIGN AND METHODS
In life dates: Not specified.
Finalisation date: 07/03/1973

Animal assignment and treatment
The test substance was suspended in corn oil and administered by a single oral gavage to 5 rats (mixed gender) per dose group at a dose level of 5010 mg/kg bw, 6310 mg/kg bw, 7940 mg/kg bw and 10000 mg/kg bw. All animals were observed for clinical signs of toxicity for a period of 7 days. At study termination all animals were sacrificed and subjected to a gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality was observed in lowest dose group (5010 mg/kg bw). Mortality occurred in 1/5 rats at 6310 mg/kg bw, 2/5 at 7940 mg/kg bw and 4/5 at 10000 mg/kg bw (see table below).

The oral LD_{50} is 8300 mg/kg bw for male and female rats (CI: 7300 mg/kg bw – 9460 mg/kg bw).

Table B.6.8.1.1.2.5-1: Toxicological investigation of: CP 50435 – Lot: XHD-16 ([redacted], 1973): 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
5.010	2, 4	1, 3, 5	-	-
6.310	6, 8, 10	7, 9	-	1/2([a])
7.940	12, 14	11, 13, 15	1/2([a])	1/3([a])
10.000	16, 18, 20	17, 19	3/3([a])	1/2([a])

- = No death
[a] Day of death not mentioned in the study report

B. CLINICAL OBSERVATIONS
Clinical signs observed included reduced appetite, reduced activity on days 1 to 3 after treatment. Furthermore, increased weakness, slight diarrhea and collapse were observed.

C. BODY WEIGHT
Body weight was not measured after treatment.

D. NECROPSY
Animals that died during the study showed slight liver discoloration and acute gastrointestinal inflammation. Animals that were sacrificed at the end of the study period showed normal appearance of the viscera by macroscopic examination.

III. CONCLUSIONS
The oral LD_{50} of the test material in rats was estimated to be 8300 mg/kg bw with a lower and upper limit of 7300 to 9460 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Based on mortality data, the LD_{50} was estimated to be 8300 mg/kg bw. However due to the very brief reporting of the experimental design and results, the study was considered as supplementary.

Assessment and conclusion by RMS:
The study is not considered acceptable for evaluation; therefore no acute oral LD_{50} is determined. The study was not accepted in the previous evaluation either (RAR, 2015).

B.6.8.1.1.3. Acute dermal toxicity

Study 1

1. Information on the study

Data point	CA 5.8.1/007
Report author	[redacted]
Report year	2002
Report title	Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration – Limit Test
Report No	16168/02
Document No	NA
Guidelines followed in study	OECD 402 (1987); Commission Directive 84/449/EEC B.3 (1992)
Deviations from current test guideline	Animals were 20-22 days old at receipt. Since no further information is given in the report, it is assumed that animals were treated after the acclimatization period of at least 5 days, i.e. animals were younger at treatment than required by the OECD guideline. The test substance was applied via a gauze which was covered by a plastic sheet, i.e. the test substance was applied under occlusive conditions instead of semi-occlusive conditions as required 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:** 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. As the deviations reported above are considered minor deviations only and not having an impact on the study outcome, the study is considered acceptable.

2. Full summary

Executive Summary

Aminomethyl phosphonic acid (AMPA) was evaluated for its acute dermal toxicity potential in male and female rats when administered at a dose level of 2000 mg/kg bw. No mortality occurred during the observation period and no clinical signs were observed at necropsy following the observation period. With regard to skin reactions, no erythema or oedema were observed during the study. 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 determined to be: LD_{50}, dermal, male and female rats > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethyl phosphonic acid (AMPA)
Identification:	AMPA
Description:	White powder
Lot/Batch #:	FA005563
Purity:	98.0 %
Stability of test compound:	Stable until December 31st, 2004
2. Vehicle and/ or positive control:	0.5 % aqueous hydroxypropylmethyl cellulose gel

3. Test animals:

Species:	Rat
Strain / Stock:	CD® / Crl: CD®
Source:	[redacted]
Age:	20 – 22 days
Sex:	Male and female
Weight at dosing:	214 - 238 g for males; 213 - 223 g for females
Acclimation period:	At least 5 days
Diet/Food:	ssniff® R/M-H V1530 (sniff Spezialdiäten GmbH, D59494 Soet); food was discontinued approximately 16 hours before administration
Water:	Tap water, *ad libitum*
Housing:	Individually housed in Makrolon cages
Environmental conditions:	Temperature: 22 ± 3°C Rel. humidity: 55 ± 15 % 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS
In life dates: 21 October 2002 to 1 November 2002

Animal assignment and treatment
The test substance was suspended to the appropriate concentration (i.e., 2000 mg/kg bw) in 0.5 % aqueous hydroxypropylmethyl cellulose gel was use applied once for 24 hours on shaved intact dorsal skin of rats.

Table B.6.8.1.1.3.1-1: Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration – Limit Test ([redacted], 2002): animal distribution

	Dose (mg/kg bw/day)	Number of Animals Per Group	
		Males	Females
Test Group	2000	5	5

The test material was applied to the skin using gauze. The gauze was covered with a plastic sheer and secured with adhesive plaster on the application site. The exposure time was 24 hours. At the end of the exposure period, possible residual substance was removed. Observations for mortality were made daily. Observations for clinical signs of toxicity were made before and immediately after application. Additional observations were made 5, 15, 30, and 60 minutes after application, as well as 3, 6, and 24 hours after application. Surviving animals were observed for a total of 14 days. During the two-week follow up period, changes in skin and fur, eyes and mucous membranes, and the respiratory, circulatory, autonomic and central nervous system and somatomotorical activity and behaviour pattern were observed at least once a day until all symptom subsided, thereafter each working day. Attention was also given to possible tremor, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Skin was observed for signs of erythema and oedema. Individual body weights were recorded prior to dosing and in weekly intervals thereafter. At the end of the study, all surviving animals were sacrificed, dissected, and inspected macroscopically. Gross pathological changes were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study. No influence on animal behaviour were noted. 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 dermal LD_{50} of the test material (AMPA) in rats was estimated to be greater than 2000 mg/kg bw.

2. Assessment and conclusion

Assessment and conclusion by applicant:
In a GLP study conducted in accordance with OECD 402 guidelines (1987), the acute dermal LD_{50} for AMPA was determined to be greater than 2000 mg/kg bw in male and female rats. The study is considered acceptable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion.
The study was also accepted in the previous evaluation (RAR, 2015).

Study 2

1. Information on the study

Data point	CA 5.8.1/008
Report author	[redacted]
Report year	1993
Report title	AMPA: Acute Dermal Toxicity (Limit) Test in Rats
Report No	8764
Document No	118-GLY
Guidelines followed in study	OECD 402 (1987); Commission Directive 84/449/EEC B.3
Deviations from current test guideline (OECD 402, 2017)	Temperature and relative humidity was outside of the guideline recommendation; it was not specified whether clinical observations were measured 30 minutes after treatment; application of the test material occurred under occlusive conditions
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 2a) **Conclusion AGG:** It is not expected that the deviation with regard to temperature and relative humidity will have had a significant impact on the study outcome. The lack of clinical observations 30 minutes after treatment does not impact the LD50 values. The application via occlusive conditions can be considered as worst case and does not impact the reliability of the study. Therefore, the study is considered to be acceptable.

2. Full summary

Executive Summary

Aminomethyl phosphonic acid (AMPA) was evaluated for its acute dermal toxicity potential in male and female rats when administered at a dose level of 2000 mg/kg bw. No mortality occurred during the observation period and no clinical signs were observed at necropsy following the observation period. Skin reactions were not reported in the study. 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 determined to be: LD_{50}, dermal, male and female rats > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminomethyl phosphonic acid (AMPA)

Identification: AMPA

Description:	White powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	Distilled water
3. Test animals:	
Species:	Rat
Strain / Stock:	Sprague-Dawley
Source:	[redacted]
Age:	8-10 weeks old
Sex:	Male and female
Weight at dosing:	218-289 g for both sexes combined
Acclimation period:	7 days
Diet/Food:	Rat and Mouse No. 1 Maintenance Diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed with a maximum of 5 animals/cage in polypropylene cages
Environmental conditions:	Temperature: 18 – 21 °C Rel. humidity: 39 % Air changes: 15-20/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS
In life dates: 1 April 1992 through 15 April 1992

Animal assignment and treatment
The test substance at a concentration of 2000 mg/kg bw was use applied once for 24 hours on shaved intact dorsal skin of rats.

Table B.6.8.1.1.3.2-1: AMPA: Acute Dermal Toxicity (Limit) Test in Rats ([redacted], 1993): Animal distribution

	Dose (mg/kg bw/day)	**Number of Animals Per Group**	
		Males	Females
Test Group	2000	5	5

The test material was applied to the skin using gauze, which was moistened with distilled water. The gauze was covered with an occlusive dressing. The exposure time was 24 hours. At the end of the exposure period, residual substance was removed. Animals were observed once daily for clinical signs of toxicity and observations continued for 14 days following dosing. Body weights were recorded 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, animals were sacrificed by carbon dioxide asphyxiation and subjected to 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 weights were considered acceptable.

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

III. CONCLUSIONS
The dermal LD_{50} of the test material (AMPA) in rats was estimated to be greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In a GLP study conducted in accordance with OECD 402 guidelines (1987), the acute dermal LD_{50} for AMPA was determined to be greater than 2000 mg/kg bw in male and female rats. The study is considered acceptable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:
It is agreed that the acute dermal LD_{50} for AMPA was determined to be greater than 2000 mg/kg bw in male and female rats in this study.
This conclusion is in line with the previous evaluation (RAR, 2015).

B.6.8.1.1.4. Skin and eye irritation

Study 1

1. Information on the study

Data point	CA 5.8.1/009
Report author	[redacted]
Report year	1973
Report title	Toxicological Investigation of: CP 50435 – Lot XHD - 16
Report No	Y-73-19 (Bayer)
Document No	NA
Guidelines followed in study	None followed
Deviations from current test guideline (OECD 404, 2002)	Exposure period was 24 hours not the recommended 4 hours; experimental procedures are only briefly described. Purity and stability of the test material is missing. Details on the test species such as age and housing conditions are missing.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, conducted prior to GLP
Acceptability/Reliability	**Conclusion GRG:** Supportive (Category 3a) **Conclusion AGG:** The study is not considered acceptable since it was not conducted according to GLP and substantial information is missing in the study report. In addition, several deviations from the OECD guidelines are noted.

2. Full summary
Aminomethyl phosphonic acid (AMPA) was tested for its dermal irritating potential. In an *in vivo* study, the 0.5 grams of the test material was applied as a finely ground powder moistened with water to the intact skin of male and female rabbits. The exposure period was 24 hours. The average maximum score was 0.0 out of a possible 8 during the seven-day observation period. Thus, indicating no skin irritating potential.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminomethyl phosphonic acid (AMPA)

Identification: Not reported

Description: Not reported

Lot/Batch #:	XHD - 16
Purity:	Not reported
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	None used
3. Test animals:	
Species:	Rabbits
Strain:	Albino
Source:	Not reported
Age:	Not reported
Sex:	Male and female
Weight at dosing:	Not reported
Acclimation period:	Not reported
Diet/Food:	Not reported
Water:	Not reported
Housing:	Not reported
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported Light/dark cycle not reported

B. STUDY DESIGN AND METHODS
In life dates: Not reported

Animal assignment and treatment
Animal assignment was not reported in the study report. The test material (0.5 grams) was applied to intact rabbit skin as a finely ground powder moistened with water. The exposure period was 24 hours.

II. RESULTS AND DISCUSSION

A. MORTALITY
Mortality was not reported.

B. CLINICAL OBSERVATIONS
Clinical signs of toxicity were not reported.

C. BODY WEIGHT
Body weights were not reported.

D. NECROPSY
A necropsy performance was not reported.

E. SKIN REACTIONS
No skin reactions were observed after 24 hours of exposure during the 7-day observation period.

III. CONCLUSIONS
Based on the EU classification criteria, AMPA is not to be classified for skin irritation.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is considered supplementary information only as just basic information is given and no individual skin scores are provided. The treatment period was 24 hours instead of 4 hours according to the most recent guideline. Therefore, the result can be considered as worst case. Based on the results of the study, no skin reactions were observed after 24 hours of exposure during the 7-day observation period. Therefore, the test

material is not considered to be irritating to the skin.

Assessment and conclusion by RMS:
The study is not considered acceptable for evaluation; therefore no conclusion on skin irritation is determined. The study was not accepted in the previous evaluation either (RAR, 2015).

Study 2

1. Information on the study

Data point:	CA 5.8.1/010
Report author	[redacted]
Report year	1973
Report title	Toxicological Investigation of: CP 50435 – Lot XHD - 16
Report No	Y-73-19
Document No	NA
Guidelines followed in study	None
Deviations from current test guideline (OECD 405, 2017)	Individual scores for eye irritation not included in the report, therefore no mean values could be obtained. Purity and stability of the test material is missing. Details on the test species such as age and housing conditions are missing.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, conducted prior to GLP
Acceptability/Reliability:	**Conclusion GRG:** Supportive (Category 3a) **Conclusion AGG:** The study is not considered acceptable since substantial information is missing in the study report. In addition, several deviations from the OECD guidelines are noted.

2. Full summary

Executive summary
In an eye irritation study, three albino rabbits were treated with glyphosate's metabolite. Therefore, the test substance was instilled into the conjunctival sac of all animals. Following treatment, eye irritation examined at 1, 24, 48, 72, 120, and 168 hours. Application of the test substance into the rabbit eye resulted in effects on the conjunctivae (slight to moderate erythema), which all resolved within 120 hours. The individual mean irritation scores could not be calculated as no individual irritation scores were given in the study report.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate metabolite CP 50435
Description:	Not specified
Lot/Batch #:	XHD – 16
Purity:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	Not specified
Source:	Not specified

Age:	Not specified
Sex:	Male and Female
Body weight at dosing:	Not specified
Acclimation period:	Not specified
Diet/Food:	Not specified
Water:	Not specified
Housing:	Not specified
Environmental conditions:	Not specified

B. STUDY DESIGN AND METHODS

In life dates: Not specified
Finalisation date: 07/03/1973

Animal assignment and treatment
An amount of 100 mg of the test substance was placed into the conjunctival sac of one eye of each animal.
At 1, 24, 48, 72, 120, and 168 hours all eyes were examined for signs of irritation.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

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

C. EYE OBSERVATIONS
Signs of oedema, erythema and discharge were seen in all animals. However, all observations had resolved until 120 hours after treatment (see below).

Table B.6.8.1.1.4.2-1: Eye irritation in rabbits after application of 'CP 50435' – Lot: XHD-16 ([redacted], 1973): Eye irritation observations

Animal number		10 min	1 h	24 h	48 h	72 h	120 h	168 h
Cornea	1-3	-	-	-	-	-	-	-
Iris	1-3	-	-	-	-	-	-	-
Conjunctiva Redness	1-3	Slight erythema	Slight erythema	Slight to moderate erythema	Slight to moderate erythema	Slight erythema in one instance	-	-
Conjunctiva Chemosis	1-3	Very slight oedema	Very slight oedema	No oedema	No oedema	No oedema	-	-
Conjunctiva Discharge	1-3	Copius discharge	Copius discharge	Moderate discharge containing whitish exudate	No discharge	No discharge	-	-

III. CONCLUSIONS
Effects on the conjunctiva were observed in all animals. These effects had resolved until 120 hours in all animals. The compound was classified as a slight eye irritant in male and female rabbits.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The classification criteria laid down in the CLP regulation (EC No. 1272/2008) could not be applied to classify the test substance, based on the given study report as there were no individual values given. However, it can be

concluded that the test substance is not corrosive as all observations on the eye were reversible within 120 hours after test item application. Detailed reporting of individual animal data is missing, therefore the study is considered as supplementary.

Assessment and conclusion by RMS:
The study is not considered acceptable for evaluation; therefore conclusion on eye irritation is determined. The study was not accepted in the previous evaluation either (RAR, 2015).

B.6.8.1.1.5. Skin sensitisation

Study 1

1. Information on the study

Data point	CA 5.8.1/011
Report author	[redacted]
Report year	2002
Report title	Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test)
Report No	16169/02
Document No	NA
Guidelines followed in study	OECD 406 (1992); Commission Directive 84/449/EEC B.6 (1996)
Deviations from current test guideline	Animals were 22 days old at start of administration, i.e. animals are not considered 'young adults' as stated in 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:** 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. As the deviations reported above are considered minor deviations only and not having an impact on the study outcome, the study is considered acceptable.

1. Full summary

Aminomethyl phosphonic acid (AMPA) 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 5 % dilution of the test item in a*qua ad iniectabilia*. The epidermal induction was conducted under occlusion on SLS-exposed skin with the test item at 50 % one week after the intradermal induction. Two weeks after the topical induction the animals were challenged by epidermal application of the test item at 50 % under occlusive dressing.
The study was performed using one control group consisting of 5 animals, and one test group consisting of 10 animals. None of the animals exhibited a positive skin reaction after the challenge treatment.

II. MATERIALS AND METHODS

A: Materials

1. Test material: Aminomethyl phosphonic acid (AMPA)

Identification: AMPA

Description: White solid powder

Lot/Batch #: FA005563

Purity:	98 %
Stability of test compound:	Expires December 31st, 2004
2. Vehicle and/ or positive control:	*Aqua ad iniectabilia* / benzocaine
3. Test animals:	
Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	[redacted]
Age:	22 days
Sex:	Male
Weight at dosing:	252 – 307 g (positive control 228 – 341 g)
Acclimation period:	At least 5 days
Diet/Food:	ssniff® Ms-H (ssniff Spezialdiäten GmbH, D-59494 Soest), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed in pairs in Makrolon cages
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 55 ± 15 % Air changes: not reported 12 hours light/dark cycle

B: Study design and methods
In life dates: 21 October 2002 to 26 November 2002

Animal assignment and treatment
AMPA was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Male Dunkin Hartley guinea pigs with body weights ranging from 252 to 307 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 10 test animals.

Table B.6.8.1.1.5.1-1: Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test) ([redacted], 2002): animal distribution

	Number of Animals Per Group
Pretest	
Intradermal Pretest	2
Epidermal Pretest	6
Main Study	
Negative Control Group	5
Test Group	10
Positive Control Group[#]	20

[#] The positive control group was not tested concurrently with the study but in a study performed in August/September 2002 in the laboratory.

The induction phase consisted of 3 injections to the intra-scapular region beginning at the cranial and ending at the caudal limits. Each animal received 2 intracutaneous injections each of: (i) 0.1 mL Freund's Complete Adjuvant (FCA) (diluted 1:1 with 0.9 % sodium chloride); (ii) 0.1 mL of the test material suspended in *aqua ad iniectabilia*; and (iii) 0.1 mL of a mixture of the test material and FCA (1:1). The third injection consisted of a final concentration of the test substance that was equal to that in the second injection. Six days after the injection phase, the application area was shaved and exposed skin was coated with 0.5 mL sodium lauryl sulfate 10 % in Vaseline in order to induce local irritation. On Day 7, an epidermal application was made and 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.

Two weeks after the topical indication, test and control animals were challenged with an occlusive patch containing the test material at a concentration of 50 %.
For control animals, topical applications used the same procedures as those noted for test animals except that the vehicle (i.e., *aqua ad iniectabilia*) alone was applied. During the challenge phase, the left flank of vehicle control animals was treated with the test material and the right flank was treated with vehicle. Positive control animals were treated with 2 % (w/v) benzocaine solution intracutaneously during the intradermal stage of induction. A 5 % (w/v) benzocaine solution was topically applied during the epidermal stage of induction and during the challenge.
Skin reactions were evaluated at 25 and 28 hours during the intradermal stage of induction and again at 49 and 72 hours during the epidermal stage of induction. Mortality and clinical signs of toxicity were observed daily during the observation period. Body weights were measured at the start of the study and at study termination. Body weights were statistically analysed suing the Student's t-test ($p \leq 0.01$).
Any animal showing erythema at the site of challenge was considered to have shown a positive response.

II. RESULTS

A. MORTALITY
There were no mortalities during the study.

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
No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group.

Table B.6.8.1.1.5.1-2: Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test) ([redacted], 2002): number of animals with positive signs following challenge

	Challenge	
	48 hours	**72 hours**
Test group (50% test material)	0/10	0/10
Negative control group	0/5	0/5
Positive control group (5% benzocaine)	20/20	20/20

III. STUDY CONCLUSION

Based on the results of the study, AMPA is not considered a skin sensitiser.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In a study conducted in accordance with OECD 406 guidelines, AMPA is not considered a skin sensitiser based on the results of a guinea pig maximisation test. The study is considered acceptable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:
It is agreed with the applicant that AMPA is not considered a skin sensitiser based on the results of this guinea pig maximisation test. The study was accepted in the previous evaluation (RAR, 2015).

Study 2

1. Information on the study

Data point	CA 5.8.1/012
Report author	[redacted]

Report year	1993
Report title	AMPA: Magnusson-Kligman Maximisation Test in Guinea Pigs
Report No	8765
Document No	NA
Guidelines followed in study	OECD 406 (1982); Commission Directive 84/449/EEC B.6 (1989)
Deviations from current test guideline (OECD 406, 1992)	In the study report it is stated that the reliability of the test system is regularly checked, however, results of these tests are not shown in the 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 to be acceptable. Although it would have been desirable to include the results from the positive control reliability tests in the study report as well, the outcome of the study is not expected to be significantly impacted.

2. Full summary

Executive summary

Aminomethyl phosphonic acid (AMPA) was tested for its sensitizing 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% concentration of the test item in 0.5% carboxymethylcellulose (CMC). The epidermal induction was conducted under occlusion with the test item at 25% in 0.5% CMC one week after the intradermal induction. Skin was treated with SLS one day before the topical induction. Two weeks after the topical induction the animals were challenged by epidermal application of the test item at 25% in 0.5% CMC.

The study was performed using one control group consisting of 20 animals, and one test group consisting of 20 animals. The sensitivity of the strain used was assessed at 6 monthly intervals using a known skin sensitizer e.g. 2,4-dinitro-chlorobenzene. None of the animals in the control and test group exhibited a positive skin reaction after the challenge treatment. Animals treated with the positive control exhibited a skin sensitizing reaction in all animals. Therefore, there is no evidence from the test results that AMPA is a skin sensitizer in guinea pigs.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethyl phosphonic acid (AMPA)
Identification:	AMPA
Description:	White solid powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	0.5 % Carboxymethylcellulose (CMC) / 2,4-dinitrochlorobenzene
3. Test animals:	
Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	[redacted]
Age:	Less than one year old
Sex:	Female
Weight at dosing:	379-484 g

Acclimation period:	At least 7 days
Diet/Food:	FDI Guinea Pig Diet, supplied by Special Diets Services, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed 5 per cage in aluminium cages
Environmental conditions:	Temperature: 19-21 °C Humidity: 44 % Air changes: not reported 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS
In life dates: 1 April 1992 through 1 May 1992

Animal assignment and treatment
AMPA 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 379 to 484 grams 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.

Table B.6.8.1.1.5.2-1: AMPA: Magnusson-Kligman Maximisation Test in Guinea Pigs ([redacted], 1993): Animal distribution

	Number of Animals Per Group
Pretest	
Intradermal Pretest	2
Epidermal Pretest	2
Main Study	
Negative Control Group	20
Test Group	20
Challenge Dose Ranging Group	4

The induction phase consisted of 6 injections consisting of two lines of three injections on each side of and parallel to the mid-line. The injections consisted of 0.1 mL Freund's Complete Adjuvant (FCA) (anterior injection), 0.1 mL test material (middle injection), and 0.1 mL of 50:50 emulsion of test material in Freund's Complete Adjuvant (posterior injection). The test material was injected at a concentration of 10% w/v in CMC. The concentration of AMPA in Freund's Complete Adjuvant was also 10%. Six days after the injection phase, the application area was shaved and exposed skin was wetted with 10% aqueous sodium lauryl sulfate in order to induce local irritation. On Day 7, an epidermal application was made and the test material was topically applied at a concentration of 25% w/v in CMC to the same pre-treated area and covered with an occlusive dressing, which was left in place for 48 hours.
Two weeks after the topical induction, test and control animals were challenged with an occlusive patch containing the test material at a concentration of 25% w/v in CMC and with vehicle (i.e., CMC). Patches were held in place for 24 hours using the same method as topical induction.

Note RMS: For challenge, the test material was selected at a concentration of 25%. The applicant is kindly asked to provide an argumentation why a higher concentration was not tested, also taking into account that higher concentrations were achieved in other studies (CA 5.8.1/011; Leuschner, 2002).

The 4 animals intended for the dose ranging for challenge were each administered 0.1 mL intradermal injection of Freund's Complete Adjuvant on either side of the mid-line of the shaved scapular region. During the challenge period, these animals remained untreated.
For the control group, animals were treated similarly to test animals but were administered CMC instead of the test material.
According to the study report, the sensitivity of this strain of guinea pig to a known sensitizer, 2,4-dinitrochlorobenzed, is checked at 6 month intervals. The most recent positive control test was completed on 23 December 1991, in which 67% of the test group reacted positively.
Body weights were measured at the start of the study and at study termination. Clinical signs of toxicity also were recorded.
Any animal showing erythema at the site of challenge was considered to have shown a positive response.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
Clinical signs of toxicity were limited to pale extremities in one test group animal.

C. BODY WEIGHT
Body weights were considered acceptable.

D. NECROPSY
A necropsy was not performed.

E. SKIN REACTIONS
No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group.

Table B.6.8.1.1.5.2-2: AMPA: Magnusson-Kligman Maximisation Test in Guinea Pigs ([redacted], 1993): Number of animals with positive signs following challenge

	Challenge at 25 %	
	24 hours	**48 hours**
Test Group	0/20	0/20
Negative Control Group	0/20	0/20
Challenge Dose Ranging Group	0/4	0/4

III. CONCLUSIONS
There is no evidence from the test results that AMPA is a skin sensitiser in guinea pigs. AMPA is not classified as a skin sensitiser according to the Magnusson-Kligman classification.

Assessment and conclusion by applicant:
In a study conducted in accordance with OECD 406 guidelines, AMPA is not considered a skin sensitiser based on the results of a guinea pig maximisation test. The study is considered acceptable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:
It is agreed with the applicant that AMPA is not considered a skin sensitiser based on the results of this guinea pig maximisation test.
The study was accepted in the previous evaluation (RAR, 2015).

B.6.8.1.1.6. Short-term toxicity

Study 1

Data point	CA 5.8.1/013
Report author	[redacted]
Report year	1978
Report title	Fourteen Day Rat Feeding
Report No	401-026
Document No	NA
Guidelines followed in study	No guideline followed.
Deviations from current test guideline (OECD 407, 2008)	No guidelines exist for 14 day feeding studies. When compared with OECD 407 (28 day oral toxicity) there was an absence of details for housing conditions and the certificates of analysis were not presented. In addition, stability and the certificate of analysis for the test substance were not

	identified. Oestrus cycle of females was not determined at necropsy. Haematology and clinical chemistry were not investigated. Full detailed gross necropsies and histopathology were not performed.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No formal claim of compliance with GLP or specific guidelines since the study was performed pre-GLP.
Acceptability/Reliability	**Conclusion AGG:** Supportive, Category 3a **Conclusion AGG:** Considering the uncertainties regarding the test material and the limited parameters investigated the study is considered to be unacceptable.

2. Full summary
Executive summary

In a 14 day dietary study in Charles River CD rats, the test material (CP 50435) was administered at dosage levels of 1000, 2000 and 4000 mg/kg bw/day. Five male and 5 female rats were used at each dose level and also in the control group. Rats were observed twice daily for overt signs of toxicity or mortality. Detailed observations were recorded weekly. Individual body weights and food consumption were recorded weekly.

Red coloured material in the urine was noted for 1 male rat at the 4000 mg/kg bw/day dosage level. No other changes were seen in general behaviour and appearance.

Male rats at the 4000 mg/kg bw/day dosage level showed slight to moderate decreases in body weight gain when compared with control and other treated rats. Food consumption was very slightly decreased for rats at the 4000 mg/kg bw/day dosage level.

None of the rats died during the study.

No gross lesions considered to be related to compound intake were seen at necropsy.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	"CP 50435; Aminomethyl Phosphonic Acid; Notebook Page 1219342-B; 99.9 % assay 7/7/77"
Description:	White granular material with a few yellowish crystalline chunks
Lot/Batch #:	Not specified
Purity:	99.9 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Purina® Laboratory Chow® / none

3. Test animals:

Species:	Rat
Strain:	Charles River CD®
Source:	[redacted]
Age:	Not specified.
Sex:	Male and female
Weight at dosing:	♂ 90 – 113 g; ♀ 85 – 98 g
Acclimation period:	Not specified.

Diet/Food:	Purina® Laboratory Chow®, *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Individual housing in hanging wire-mesh cages	
Environmental conditions:	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Light cycle:	Not reported

B. STUDY DESIGN AND METHODS

In life dates: 1977-11-25 (start of dosing) to 1977-12-09 (terminal necropsies)

Animal assignment and treatment

The test material (CP 50435) was administered in the diet at varying concentrations to provide dosage levels of 1000, 2000 and 4000 mg/kg bw/day. Five male and 5 female rats were used at each dose level and also in a control group. Control rats received the basal diet, Purina® Laboratory Chow® on the same regimen as treated rats.

Table 6.8.1.1.6.1-1: Fourteen Day Rat Feeding Study [redacted]-77-309 ([redacted], 1978): Study design

Test group	Dose Group [mg AMPA/kg bw/day]	Number of Rats	
		Males	Females
Control	0	5	5
Low	1000	5	5
Intermediate	2000	5	5
High	4000	5	5

The compound was ground in a mortar with pestle prior to weighing. The appropriate amount of compound was then mixed with a small amount of basal diet in a Hobart food mixer. This premix was mixed with the total amount of diet in a twin shell blender (with an intensifier bar). The diets were mixed weekly.

Diet Samples

A sample of the compound of approx. 42.5 g (equivalent to 1.5 ounce) was taken at the start of the study and at the end of the study. Weekly samples of control feed and each treatment diet were taken on day 1 of feeding. Additional containers with feed from each diet level were placed in an empty cage at the beginning of each feeding week and sampled at the end of the week. The diet samples were immediately frozen for shipping to the sponsor after termination of the study.

Mortality

The animals were observed twice daily for mortality and signs of overt toxicity.

Clinical observations

Detailed observations of each rat were recorded weekly.

Body weight

Individual body weights were recorded weekly.

Food consumption

Individual food consumption was recorded weekly.

II. RESULTS AND DISCUSSION

A. GENERAL BEHAVIOUR, APPEARANCE AND SURVIVAL

Red coloured material in the urine was noted for 1 male rat at the 4000 mg/kg bw/day dosage level.
No other changes were seen in general behaviour and appearance. No rats died during the study.

B. BODY WEIGHT

Male rats at the 4000 mg/kg bw/day dosage level showed slight to moderate decreases in body weight gain when compared with control and other treated rats. Changes in body weight were similar for control and treated female rats. Group mean body weights at study initiation and termination are presented below (see table below).

Table 6.8.1.1.1.6.1-2: Fourteen Day Rat Feeding Study [redacted]-77-309 ([redacted] 1978): Intergroup comparison of group mean body weights and body weight gain

Dosage Group [mg/kg bw/day]	Mean body weight [g]		
	Week 0	Week 2	Percentage Increase [%]
	Males		
0 (control)	99 (100%)	193 (100%)	94.9
1000	101 (102%)	203 (105%)	101.0
2000	103 (104%)	193 (100%)	87.4
4000	105 (106%)	183 (95%)	74.3
	Females		
0 (control)	94 (100%)	152 (100%)	61.7
1000	92 (98%)	149 (98%)	62.0
2000	95 (101%)	156 (103%)	64.2
4000	91 (97%)	143 (94%)	57.1

C. FOOD CONSUMPTION

Food consumption was very slightly decreased for rats at the 4000 mg/kg bw/day dosage level. Group mean food and compound consumptions for the study are presented below:

Table 6.8.1.1.1.6.1-2: Fourteen Day Rat Feeding Study [redacted]-77-309 ([redacted], 1978): Intergroup comparison of mean food consumption and test substance intake

Dosage Group [mg/kg bw/day]	Average Food Consumption [g/rat/day]		Compound Consumption [mg/kg bw/day]	
Males	**Week 1**	**Week 2**	**Week 1**	**Week 2**
0 (control)	19.7 (100%)	23.4 (100%)	-	-
1000	20.6 (105%)	24.2 (103%)	1068	1090
2000	20.1 (102%)	24.2 (103%)	2195	2232
4000	18.5 (94%)	21.1 (90%)	4152	4337
Females				
0 (control)	16.4 (100%)	19.1 (100%)	-	-
1000	16.9 (103%)	19.2 (101%)	1063	1059
2000	17.4 (106%)	19.0 (99%)	2139	1990
4000	15.6 (95%)	17.9 (94%)	4070	4303

D. NECROPSY FINDINGS

Raised, opaque corneal foci were seen in one male rat of the Low dose group and hydrometra was observed in the uterus of one female rats of the Intermediate group. Both findings were considered to be of no toxicological relevance.

There were no other treatment-related gross pathological findings.

Assessment and conclusion by applicant:

In this 14 day dietary study in Charles River CD rats, the test material AMPA was administered at dosage levels of 1000, 2000 and 4000 mg/kg bw/day.

The study was not conducted according to any guideline or in compliance with GLP (pre-GLP). Therefore, the major deviations listed above are from the current version of OECD 407 (2008): no haematology, no clinical chemistry, no full detailed gross necropsies and histopathology.

Apart from these major deviations, the study was well conducted and can provide supplemental information for the assessment of the metabolites of glyphosate. The study is therefore considered to be only supplementary.

Dosing CD rats with AMPA via the diet did not produce any changes in general behaviour or appearance. Slight to moderate decrease in body weight gain was observed in males at 4000 mg/kg bw/day. Food consumption was very slightly decreased in male and female rats at the 4000 mg/kg bw/day dose level. One male rat at the high dose showed red coloured material in the urine.

No gross lesions considered to be compound related were observed. Due to the absence of relevant examinations such as haematology, clinical chemistry or histopathology, no reliable NOAEL could be derived from this study.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. Due to the severe reporting deficiencies and limitations of the study it is not considered acceptable. This conclusion is in line with the previous assessment (RAR, 2015)

Study 2

Data point	CA 5.8.1/014
Report author	[redacted] *et al.*
Report year	1993
Report title	4 Week Dose Range Finding Study in Rats with Administration by Gavage
Report No	7803
Document No	148-GLY
Guidelines followed in study	No guideline followed.
Deviations from current test guideline	This study was essentially a dose range-finding study for which there are no guidelines. Haematology and clinical biochemistry parameters were not measured or evaluated. General gross pathology of organs and tissues was not performed - only selected organs and tissues were weighed, fixed or examined as detailed below. Histopathology was only performed on urinary bladder, mandibular salivary gland, sublingual salivary gland and parotid salivary gland.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion AGG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable but with restrictions (reliable with restrictions). Considering it is a dose range finding study the limitations are not considered to impact the outcome of the study.

Executive summary

Groups of 5 male and 5 female Sprague-Dawley rats were dosed orally via a steel dosing cannula with AMPA (aminomethylphosphonic acid) at dose levels of 0, 10, 100, 350 or 1000 mg/kg bw/day.
After 4 weeks of dosing all surviving animals were killed, subjected to necropsy and had selected organs weighed.
All animals underwent histological examinations of the urinary bladder, mandibular salivary gland, sublingual salivary gland and parotid salivary gland.
The results are summarised as follows:

Mortality:	There were no premature decedents.
Clinical Signs:	There were no clinical signs.
Body Weight:	There were no notable intergroup differences.
Food Consumption:	There were no notable intergroup differences.
Water Consumption:	There were no notable intergroup differences.
Organ weights:	There was a slight equivocal increase in kidney weight at 350 and 1000 mg/kg bw/day in males.
Necropsy Findings:	There were no notable intergroup differences.
Histological Findings:	There were no notable intergroup differences.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA (Aminomethylphosphonic acid)
Description:	White Powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	The test substance is stable at least 3 years from the date of analysis at ambient temperature in the dark.
2. Vehicle and/ or positive control:	0.5 % Carboxymethylcellulose (CMC) in distilled water / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 4 weeks
Sex:	Male and female
Weight at dosing:	♂ 209 – 230 g; ♀ 156 – 162 g
Acclimation period:	16 days
Diet/Food:	SQC Expanded Maintenance Diet No. 1 Rat and Mouse (pelleted), *ad libitum*,
Water:	Tap water, *ad libitum*
Housing:	2 of one sex or 1 animal/cage in polypropylene cages with stainless steel wire grid tops and bottoms (420 × 270 × 200 mm).
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 50 ± 15 % Air changes: 15 – 20 / hour 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1992-02-20 (start of dosing) to 1992-03-19 (terminal necropsies)

Animal assignment and treatment

In a 4 week dose range finding study groups of 5 Sprague-Dawley rats per sex received daily doses of 0, 10, 100, 350 or 1000 mg/kg bw/day, orally via steel dosing cannula, at a dose volume of 10 mL/kg bw for 28 consecutive days. The dose formulations were prepared fresh daily using 0.5 % carboxymethylcellulose (CMC) in distilled water as vehicle. Samples of formulations for all dosing groups (including Control) were analysed during Weeks 1 and 4 of dosing for the concentration of test item in the suspension. In addition, data demonstrating 24 hour stability of dosing suspensions were generated during the study.

Table 6.8.1.1.6.2-3: 4 Week Dose Range Finding Study in Rats with Administration by Gavage ([redacted] 1993): Study design

Test group	Dose Group [mg AMPA/kg bw/day]	Animal Numbers	
		Males	Females
Control	0	1 – 5	26 – 30
Low	10	6 – 10	31 – 35
Intermediate I	100	11 – 15	36 – 40
Intermediate II	350	16 – 20	41 – 45
High	1000	21 – 25	46 – 50

Mortality
All animals were checked for viability/mortality early each morning and as late as possible each day.

Clinical observations
All animals were examined for signs of reaction to treatment each day. All animals received a detailed clinical examination once each week.

Body weight
The body weight of each animal was recorded on three occasions over a 10 day pre-trial period and daily from the start of treatment until the end of the study.

Food consumption
The quantity of food consumed by each cage of animals was recorded on three occasions over a 10 day pre-trial period and twice each week from the start of treatment until the end of the study.

Water consumption
Water consumption was monitored by visual inspection throughout the treatment period.

Terminal studies
After 28 consecutive days of treatment, all surviving animals were killed by carbon dioxide asphyxiation followed by exsanguination. Gross dissection and necropsy were performed under the supervision of a pathologist.

The following organs were weighed and fixed: Liver, heart, kidneys, lung, spleen, testes, ovaries, mandibular salivary gland, sublingual salivary gland and parotid salivary gland.

The following organs were fixed and examined histologically: Urinary bladder, mandibular salivary gland, sublingual salivary gland and parotid salivary gland.

The following organs were fixed: Abnormal tissue and ears.

The above tissues were fixed in 10 % neutral buffered formalin. Lungs were fixed in their entirety by perfusion with 10 % neutral buffered formalin.
Ears were fixed for identification purposes.

Histological evaluation of the urinary bladder, mandibular salivary gland, sublingual salivary gland and parotid salivary gland was performed for all animals.

Carcasses of all animals were discarded immediately following necropsy and placing of all tissues in fixative as identified above.

Statistics
Organ weight and body weight data were statistically analysed for homogeneity of variance using the F-max test. If group variances appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If variances were heterogeneous log or square root transformations were used in an attempt to stabilise the variances. If variances were still heterogeneous, a non-parametric test such as a Kruskal-Wallis ANOVA was used and pairwise comparisons made via Dunn Z test where considered appropriate.

Organ weights were also analysed conditional on body weight (i.e. analysis of covariance).

Histological data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no premature decedents.

B. CLINICAL OBSERVATIONS
There were no clinical signs observed throughout the dose groups in either sex.

C. BODY WEIGHT
There were no notable intergroup differences in male animals.

A slight reduction in overall group mean body weight gain (13%) was noted in High dose females (see table below), however, due to the small magnitude of difference and lack of statistical significance at any time point, this reduction was considered not to be related to treatment with AMPA.

Table 6.8.1.1.6.2-4: 4 Week Dose Range Finding Study in Rats with Administration by Gavage ([redacted], 1993): Intergroup comparison of group mean body weights and body weight gain

Time point	Mean body weight or body weight gain [g]		
	Initial body weight	Final body weight	Total weight gain Day 0 – 28
Dose [mg/kg bw/day]	**Males**		
0	215	406 (100%)	191
10	220	399 (98%)	179 (94 % of Control)
100	209	420 (103%)	211 (110 % of Control)
350	230	420 (103%)	190 (99 % of Control)
1000	220	414 (102%)	194 (102 % of Control)
	Females		
0	162	254 (100%)	92
10	159	258 (102%)	99 (108 % of Control)
100	156	244 (96%)	88 (96 % of Control)
350	162	257 (101%)	95 (103 % of Control)
1000	160	240 (94%)	80 (87 % of Control)

D. FOOD CONSUMPTION
There were no notable intergroup differences for food consumption in either sex.

E. WATER CONSUMPTION
There were no notable intergroup differences for water consumption in either sex.

F. TERMINAL STUDIES

Organ weights
In males, absolute organ weights showed no notable intergroup differences.
Following covariance analysis a slight increase in kidney weight in male animals was observed for the Intermediate II and High dose groups (7 %, $p<0.05$ and 8 %, $p<0.05$, respectively). In addition, a slight increase

in liver weight (10 %, p<0.05) was noted in the Intermediate II dose group males. However, due to an absence of similar effect observed in the High dose group, an absence of similar effects in females, no dose-response relationship and the magnitude of change, this increase was considered not to be related to treatment with AMPA and not adverse.

In females, there were no notable intergroup differences.

Table 6.8.1.1.6.2-5: 4 Week Dose Range Finding Study in Rats with Administration by Gavage ([redacted] 1993): Intergroup comparison of selected relative mean organ weights (mean ± SE)

Organ [g]	Dose Groups [mg AMPA/kg bw/day]									
	Males					Females				
	0	10	100	350	1000	0	10	100	350	1000
Kidney (covariance analysis)	3.16 ± 0.06 100%	3.26 ± 0.06 103%	3.01 ± 0.06 95%	**3.37* ± 0.06** 107%	**3.40* ± 0.06** 108%	2.03 ± 0.09 100%	2.13 ± 0.09 105%	2.16 ± 0.09 106%	2.12 ± 0.09 104%	2.19 ± 0.09 108%
Liver (covariance analysis)	17.67 ± 0.54 100%	18.91 ± 0.54 107%	17.18 ± 0.54 97%	**19.44* ± 0.54** 110%	18.06 ± 0.53 102%	10.78 ± 0.39 100%	11.10 ± 0.40 103%	10.98 ± 0.40 102%	10.41 ± 0.40 97%	9.88 ± 0.40 92%

*: Statistically significant from controls, p<0.05

Gross pathology
Enlargement of the submandibular lymph node was seen in all dose groups (including Controls) except for male animals in the Intermediate II dose group. The incidence varied and despite being highest in the High dose females (5/5 compared with 1/5 in the Control) this finding was considered not to be related to treatment with AMPA due to the lack of a dose-response relationship. There were no other notable intergroup differences.

Table 6.8.1.1.6.2-6: 4 Week Dose Range Finding Study in Rats with Administration by Gavage ([redacted] 1993): Summary of necropsy findings

Finding	Dose Groups [mg AMPA/kg bw/day]									
	Males					Females				
	0	10	100	350	1000	0	10	100	350	1000
Submandibular salivary lymph node: enlarged	3 / 5	1 / 5	2 / 5	0 / 5	1 / 5	1 / 5	3 / 5	3 / 5	3 / 5	5 / 5

Histopathology
In males, very mild reduced serous secretion in the mandibular salivary gland was noted in 1/5 animals in the high dose group. The change was seen as reduced eosinophilic droplets in the epithelial cells of the serous duct acini. The finding was confirmed by staining with the PAS-Alcian blue method for mucopolysaccharides. This very mild reduced serous secretion does not resemble salivary gland changes seen in long-term studies with glyphosate (parent compound of AMPA) and was considered not to be related to treatment with AMPA.

In females, no histological changes were seen.

Table 6.8.1.1.6.2-7: 4 Week Dose Range Finding Study in Rats with Administration by Gavage (H[redacted] 1993): Summary of histological findings

Finding	Dose Groups [mg AMPA/kg bw/day]									
	Males					Females				
	0	10	100	350	1000	0	10	100	350	1000
Salivary gland: Mandibular: reduced serous secretion	0 / 5	0 / 5	0 / 5	0 / 5	1 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5

III. CONCLUSIONS

Dosing of Sprague-Dawley rats orally by gavage for 4 weeks with AMPA did not produce in-life signs of toxicity. Organ weight analysis revealed a slight equivocal increase in kidney weight after covariance analysis at 350 and 1000 mg/kg bw/day in males. There were no toxicologically significant effects seen at 10 or 100 mg/kg bw/day.

4. Assessment and conclusion

Assessment and conclusion by applicant:

In this 4 week dose range finding study groups of 5 Sprague-Dawley rats per sex received daily doses of AMPA at 0, 10, 100, 350 or 1000 mg/kg bw/day by gavage for 28 consecutive days.
The study was conducted in compliance with GLP regulations. Deviations could not be applied as no guideline was followed.
However, the study was well conducted, in compliance with GLP and thus, can provide useful information for the assessment of the metabolites of glyphosate. The study is therefore considered valid.

Dosing of Sprague-Dawley rats orally by gavage for 4 weeks with AMPA did not produce in-life signs of toxicity. Organ weight analysis revealed a slight equivocal increase in kidney weight after covariance analysis at 350 and 1000 mg/kg bw/day in males.

There were no toxicologically significant effects seen at 10 or 100 mg/kg bw/day. Based on the slightly higher kidney weight in male rats, the NOAEL for AMPA can be set at 100 mg/kg bw/day in this dose range finding study.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable but with restrictions (reliable with restrictions), considering it is a dose range finding study. In line with the conclusion in RAR (2015) the NOAEL is set at 100 mg/kg bw/d for males and 350 mg/kg bw/d for females, based on the increased kidney weight and reduced body weight gain, respectively.

Study 3

Data point:	CA 5.8.1/015
Report author	[redacted]
Report year	1991
Report title	One Month Study of AMPA Administered by Capsule to Beagle Dogs
Report No	[redacted]-11127
Document No	NA
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Subdivision F, FIFRA, Hazard Evaluation: Human and Domestic Animals, Section 82-1 and also the OECD Toxicity Test Guidelines (ISBN 92-64-12221-4, Section 409; 1981)
Deviations from current test guideline (OECD 409, 1998)	Ophthalmology not performed. Urinalysis not investigated. The following organ weights were not determined: Gall bladder, epididymides, ovaries, uterus and thymus. No histopathological examination of relevant tissues performed (all gross lesions, liver with gall bladder, kidneys, adrenals, testes, epididymides, ovaries, uterus, thyroid (with parathyroids), thymus, spleen, brain, heart, pituitary, eyes, oesophagus, salivary glands, stomach, small and large intestines, pancreas, trachea and lungs, aorta, accessory sex organs, female mammary gland, prostate, lymph nodes, peripheral nerve and bone marrow).
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 to be unacceptable mainly due to the lack of histopathological investigations and the low number of animals per dose group.

2. Full summary

Executive summary

AMPA was administered by capsule to groups of 2 beagle dogs/sex at dosages of 0, 10, 30, 100, 300 or 1000 mg/kg bw/day. Clinical observations were performed at least weekly with additional clinical signs documented by exception. Body weights and food consumption were determined weekly. Clinicopathologic examinations including haematology and clinical blood chemistry were performed at termination (1 month). All survivors were sacrificed at termination and given a complete necropsy (including weighing of selected organs). No tissues were retained.
Haematological changes at 100 mg/kg bw/day included decreased RBC counts, increased reticulocyte counts, decreased haemoglobin and decreased haematocrit in both sexes. Haematologic changes at 300 mg/kg bw/day included decreased reticulocyte counts, haematocrit and haemoglobin in females. These changes were indicative of a mild to moderate anaemia which was of undetermined aetiology.
No other signs of toxicity were observed in this study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA (Aminomethylphosphonic acid) / EHL Substance Identification Code: T900031
Description:	White powder
Lot/Batch #:	HET-9001-1463T
Purity:	94.38 %
Stability of test compound:	Not reported.

2. Vehicle and/or positive control: Empty gelatine capsule / none

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 7.3 – 9.8 kg; ♀ 5.9 – 8.4 kg
Acclimation period:	41 days
Diet/Food:	Purina Mills Certified Canine Diet Meal #5007 (offered 1 – 2 hours each day)
Water:	Tap water, *ad libitum*
Housing:	Individual housing in stainless steel cages
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported Light cycle: Not reported

B. STUDY DESIGN AND METHODS

In life dates: 1990-04-04 (start of dosing) to 1990-05-03 (terminal necropsies)

Animal Assignment and Treatment:
AMPA was administered to groups of 2 beagle dogs/sex at dosages of 0, 10, 30, 100, 300 or 1000 mg/kg bw/day. Neat test material was put into gelatine capsules and administered orally.

Table 6.8.1-6.8.1-8: One Month Study of AMPA Administered by Capsule to Beagle Dogs ([redacted], 1991): Study design

Group Number	Dose Group [mg AMPA/kg bw/day]	Number of Animals	
		Males	Females
1	0	2	2
2	10	2	2
3	30	2	2
4	100	2	2
5	300	2	2
6	1000	2	2

No analysis was performed to verify identity, purity or stability or otherwise characterise the test material.

In-life Observations
Checks for mortality, moribundity and noteworthy signs of toxicity were performed twice daily (AM and PM; documented by exception). Detailed observations for signs of toxicity were performed once weekly.

Body Weight
Body weights were recorded prior to randomisation and once weekly thereafter.

Food Consumption
Food consumption was analysed on a weekly basis by extrapolation from four or five-day determination of actual food consumption each week.

Ophthalmoscopic Examination
Not performed.

Clinical Pathology
At termination, all animals had blood samples collected from the jugular vein for haematology and blood chemistry determinations.

Haematology
The following parameters were evaluated: Total erythrocyte count, total leukocyte count, platelet count, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and activated partial thromboplastin time (APTT).

For leukocyte differential, blood smears were prepared on glass slides, stained with Wright's stain, and examined microscopically.

For reticulocyte count, a portion of the EDTA-treated blood sample was mixed with vital stain (methylene blue) and a slide was prepared and examined microscopically.

Blood Chemistry
The following parameters were evaluated: Albumin, total protein, blood urea nitrogen, total bilirubin, direct bilirubin, glucose, glutamic pyruvic transaminase, alkaline phosphatase, glutamic oxaloacetic transaminase, gamma glutamyl transpeptidase, creatinine, cholesterol, calcium, phosphorus, chloride, sodium, potassium and globulin.

Gross Pathology
All animals were subjected to an external and internal examination. Internal cavities were opened and the organs were examined in situ and then removed. Hollow organs were opened and examined.

Organ Weights
The following organs were weighed: Adrenals, brain, heart, kidneys, liver, spleen, testes and thyroids.
No tissues were retained or fixed.

Statistics
Because there were only two animals of each sex in each treatment group, statistical analysis often could not be performed or was inappropriate. When it was, the following statistical procedures were used to detect statistically significant differences between treated animals and their respective controls:

Dunnett's Multiple Comparison Test (two-tailed): In-life body weights, cumulative body weight changes, food consumption and APTT.

EHL decision tree analysis: Haematology data, clinical chemistry data, terminal body weights, absolute organ weights and organ/body weight ratios were evaluated by decision-tree statistical analyses which, depending on the results of tests for normality and homogeneity of variances, chose either parametric or nonparametric routines to detect differences and analyse for trend.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no unscheduled deaths in this study.

B. CLINICAL OBSERVATIONS
Diarrhoea was observed in both males and one female at 1000 mg/kg bw/day (total of 18 incidents) and emesis was observed in both females and one male at 1000 mg/kg bw/day (total of 4 incidents).

C. BODY WEIGHT
There were no effects on body weight.

D. FOOD CONSUMPTION
There were no effects on food consumption.

E. CLINICAL PATHOLOGY

Haematology
Haematologic changes at 1000 mg/kg bw/day included decreased red blood cell counts, increased reticulocytes, decreased haemoglobin and decreased haematocrit in both sexes. Haematologic changes at 300 mg/kg bw/day included decreased reticulocyte counts, haematocrit and haemoglobin in females.
Statistically significant decreases in APTT in females at all dose levels were minor in magnitude and were not considered toxicologically significant.

Table 6.8.1.1.6.3-9: One Month Study of AMPA Administered by Capsule to Beagle Dogs : Intergroup comparisons of selected group mean haematology parameters

	Group Mean Haematology Parameters				
Dose Group [mg/kg bw/day]	**Total Erythrocyte Count [10^6/mm^3]**	**Reticulocytes [10^3/mm^3]**	**Haemoglobin [g/dL]**	**Haematocrit [%]**	**APPT [s]**
	Males				
0	6.3200 ± 0.1131	31.8000 ± 22.9103	14.4000 ± 0.9899	41.3000 ± 2.9689	15.9 ± 0.4
10	6.0350 ± 0.0778 (95%)	22.7825 ± 23.7623 (72%)	14.5000 ± 0.1414 (101%)	41.4000 ± 0.7071 (100%)	15.9 ± 0.4 (100%)
300	6.2600 ± 0.3818 (99%)	55.5425 ± 73.9315 (175%)	14.8500 ± 0.9192 (103%)	42.4000 ± 2.6870 (103%)	16.8 ± 0.6 (106%)
100	6.8150 ± 0.1626 (108%)	18.9425 ± 17.3135 (60%)	15.5000 ± 0.4243 (108%)	44.7500 ± 1.3435 (108%)	16.8 ± 0.6 (106%)
300	6.0750 ± 0.4031 (95%)	21.1200 ± 2.8850 (86%)	14.4500 ± 1.3435 (100%)	40.9500 ± 3.7477 (99%)	15.4 ± 0.4 (97%)
1000	**5.2000* ± 0.0424 (82%)**	71.3725 ± 30.6708 (224%)	12.5000 ± 0.1414 (87%)	35.4000 ± 0.7071 (86%)	15.0 ± 0.6 (94%)

	Females				
0	6.3600 ± 0.4384	22.1050 ± 2.9628	15.6500 ± 0.7778	44.1000 ± 1.9799	17.4 ± 0.0
10	6.7300 ± 0.0283 (106%)	20.1500 ± 18.9505 (91%)	15.7000 ± 0.0000 (100%)	44.8500 ± 0.4950 (102%)	**16.1* ± 0.1 (93%)**
30	6.5600 ± 0.1838 (103%)	23.0900 ± 9.9207 (104%)	15.2500 ± 0.3536 (97%)	43.5000 ± 1.2728 (99%)	**15.7** ± 0.4 (90%)**
100	6.1200 ± 0.4667 (96%)	23.3625 ± 12.5688 (106%)	14.6000 ± 0.0000 (93%)	41.8500 ± 0.0707 (95%)	**16.1* ± 0.1 (93%)**
300	5.6500 ± 0.0000 (89%)	7.0625 ± 1.9976 (32%)	**13.6000* ± 0.5657 (87%)**	**38.5500* ± 1.3435 (87%)**	**16.0* ± 0.0 (92%)**
1000	**5.0500* ± 0.1838 (79%)**	**101.4550** ± 28.8732 (459%)**	**12.1000** ± 0.8485 (77%)**	**34.2500** ± 2.1920 78%)**	**15.3** ± 0.7 (88%)**

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

Blood chemistry

There were no effects on blood chemistry parameters.

G. GROSS PATHOLOGY

There were no effects on organ weights and there were no necropsy observations which were considered related to administration of the test material.

3. Assessment and conclusion

Assessment and conclusion by applicant:

AMPA was administered orally (via capsule) to groups of 2 beagle dogs/sex at dosages of 0, 10, 30, 100, 300 or 1000 mg/kg bw/day for a period of one month.
The study was conducted according to OECD 408 (1981) and in compliance with GLP. However, there were major deviations to current standards: no ophthalmology, no urinalysis, no histopathology.
Apart from these major deviations, the study was well conducted and can provide supplemental information for the assessment of the metabolites of glyphosate. The study is therefore considered to be only supplementary.
There were no mortalities or treatment related effects upon clinical observations, body weight, food consumption, blood chemistry, organ weights or necropsy in either sex.
At 1000 mg/kg bw/day decreased erythrocyte counts, increased reticulocyte counts and decreased haematocrit was observed in both sexes.
At 300 mg/kg bw/day decreased reticulocyte counts, haematocrit and haemoglobin was observed in females. These changes were indicative of mild to moderate anaemia of undetermined aetiology.
No other signs of toxicity were observed in this study.
Under the conditions of this study, the No-Observed-Adverse-Effect-Level (NOAEL) was considered to be 300 mg/kg bw/day in male and 100 mg/kg bw/day in female Beagle dogs, respectively.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study has major deviations from the OECD guidance and therefore the study was not accepted in the RAR (2015). Due to the limitations it is agreed that the study cannot be used to derive a NOAEL for AMPA. Signs of toxicity were reported in this study. Haematological changes found are indicative of anaemia at 300 mg/kg bw/d in females and 1000 mg/kg bw/d in both males and females. Additionally, at the top dose level there were more frequent occurrences of diarrhoea. This conclusion is in line with the DAR.

Study 4

Data point	CA 5.8.1/016
Report author	[redacted]

Report year	1993
Report title	13 Week Toxicity Study in Rats with Administration by Gavage
Report No	7866
Document No	152-GLY
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision, FIFRA, 82-1, in general accordance with OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Functional observation and/or sensor reactivity assessments were not performed. Platelet count not included within haematology parameters. Total cholesterol, HDL, LDL and urea were not evaluated as part of clinical chemistry parameters. In addition, only 2 liver enzymes (AST and ALT) were measured. T4, T3 and TSH were not measured. Vaginal smears for oestrus cycle determination of females not performed at necropsy.
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).

2. Full summary

Executive summary

Groups of 10 male and 10 female Sprague-Dawley rats were dosed orally each day for 13 weeks with AMPA (aminomethylphosphonic acid) at dose levels of 0, 10, 100 or 1000 mg/kg bw/day via a steel dosing cannula.

Blood samples were taken from all animals during Week 13 for investigations of haematology and clinical chemistry parameters. An ophthalmoscopic examination was undertaken on all animals during pre-trial and on all Control and High dose animals during Week 12.
On completion of 13 weeks dosing all surviving animals were killed and necropsied. Premature decedents were also necropsied. Histological examination was carried out on a full list of organs from all Control and High dose animals and all premature decedents. In addition, histological examination was carried out on the kidneys, liver, lungs, submaxillary salivary gland, sublingual salivary gland and parotid salivary gland of all other animals.

There were 4 premature deaths (one intermediate dose male, one low dose female and two intermediate dose females). None of these deaths were due to administration of AMPA.

Clinical Signs: There were no notable clinical signs.
Body Weight: There were no notable intergroup differences.
Food Consumption: There were no notable intergroup differences.
Water Consumption: There were no notable intergroup differences.
Haematology: There were no notable intergroup differences.
Clinical Chemistry: There were no notable intergroup differences.
Organ weights: There were no notable intergroup differences.
Necropsy Findings: There were no necropsy findings which were considered to be due to treatment with AMPA.
Histological Findings: There were no histological findings which were considered to be due to treatment with AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: AMPA (aminomethylphosphonic acid)

Description: White Powder

Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	The test substance is stable at least 3 years from the date of analysis at ambient temperature in the dark.
2. Vehicle and/ or positive control:	0.5 % Carboxymethylcellulose (CMC) in distilled water / none
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	approx. 4 weeks (on arrival)
Sex:	Male and female
Weight at dosing:	♂ 144 – 168 g; ♀ 106 – 129 g
Acclimation period:	13 days
Diet/Food:	Rat and Mouse (modified) No. 1 Diet SQC (pelleted), *ad libitum*, (except for laboratory investigation sampling during Week 13 where animals were deprived of food overnight)
Water:	Tap water, *ad libitum*
Housing:	2 of one sex per cage in polypropylene cages with stainless steel wire grid tops and bottoms (420 × 270 × 200 mm).
Environmental conditions:	Temperature: 20 °C Humidity: 50 ± 15 % Air changes: 15 – 20 / hour 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1992-04-16 (start of dosing) to 1992-07-17 (terminal necropsies)

Animal assignment and treatment:
In a 13 week oral toxicity study groups of 10 Sprague-Dawley rats per sex received daily doses of 0, 10, 100 or 1000 mg/kg bw/day, via gavage, at a dose volume of 10 mL/kg bw. The dose formulations were prepared fresh daily using 0.5 % carboxymethylcellulose (CMC) in distilled water as vehicle. Samples of formulations for all dosing groups (including Control) were analysed during Weeks 1, 6 and 13 of dosing for the concentration of test item in the suspension.

Table 6.8.1.1.6.4-10: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted] 1993): Study design

Test group	Dose Group [mg AMPA/kg bw/day]	Animal Numbers	
		Males	Females
Control	0	1 – 10	41 – 50
Low	10	11 – 20	51 – 60
Intermediate	100	21 – 30	61 – 70
High	1000	31 – 40	71 – 80

Mortality
All animals were checked for viability/mortality early each morning an as late as possible each day.

Clinical observations
All animals were examined for signs of reaction to treatment each day. All animals received a detailed clinical examination once each week.

Body weight

The body weight of each animal was recorded once each week before treatment and weekly from the start of treatment until the end of the study.

Food consumption
The quantity of food consumed by each cage of animals was recorded once each week before treatment and weekly from the start of treatment until the end of the study.

Water consumption
Water consumption was monitored by visual inspection throughout the treatment period.

Ophthalmoscopic examination
The eyes of all animals from all dose groups were examined during pretrial and of all Control and High dose animals during Week 12 of dosing. Ophthalmoscopic examinations of the anterior, lenticular and fundic areas were evaluated.

Haematology and clinical chemistry
Samples for laboratory investigation were taken from all animals during Week 13 of dosing after overnight deprivation of food. Samples for haematology were measured on whole blood taken into tubes containing EDTA. Samples for clinical chemistry were measured from plasma of whole blood taken into heparin. The following haematological parameters were measured: Haemoglobin (Hb), red blood cell count, haematocrit, white blood cell count, clotting time, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells.

For clinical chemistry analysis the following parameters were measured: blood urea nitrogen, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium, potassium, chloride, total protein, albumin, albumin/globulin ratio, creatinine, calcium, phosphate and total bilirubin.

Terminal studies

After 13 weeks of consecutive treatment, all surviving animals were killed by carbon dioxide asphyxiation followed by exsanguination. Gross dissection and necropsy were performed under the supervision of a pathologist.

The following organs were weighed, fixed and examined histologically: Liver, heart, kidneys, lung, spleen, adrenal glands, thymus, testes, ovaries, prostate, uterus, brain, submaxillary (mandibular) salivary gland, sublingual salivary gland, parotid salivary gland and pituitary.

The following organs were fixed and examined histologically: Seminal vesicles, vagina, cervix uteri, spinal cord, thigh muscle, pancreas, submandibular lymph node, skin and mammary gland, urinary bladder, eyes, optic nerves, tongue, aortic arch, mesenteric lymph node, thyroids, parathyroids, trachea, salivary gland (submaxillary (mandibular), sublingual and parotid), oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, sciatic nerve, sternum and rib and any abnormal tissue.

The following organs were fixed: Rectum, smooth muscle (large intestine), nasal cavity and ears.

The above tissues were fixed in 10 % neutral buffered formalin. Lungs were fixed in their entirety by perfusion with 10 % buffered formalin. Testes were weighed with epididymides. Submaxillary and sublingual salivary glands were weighed together. Contracted bladders were distended with fixative with the epithelial surface examined after fixation. Optic nerves and eyes were fixed in Davidson’s fluid. Thyroids (with parathyroids) were weighed after fixation. Ears were fixed for identification purposes.

Histological evaluation was performed on all animals from the Control and High dose groups in addition to any premature decedents. In addition, kidneys, livers, lungs, submaxillary salivary glands, sublingual salivary glands and parotid salivary glands were examined from the Low and Intermediate dose groups.

The stomach and intestines were opened at necropsy and the mucosal surface examined. Carcasses of all animals were discarded immediately following necropsy and placing of all tissues in fixative as identified above.

Statistics

Body weight, haematology, clinical chemistry and organ weight data were statistically analysed for homogeneity of variance using the F-max test. If group variances appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If variances were heterogeneous log or square root transformations were used in an attempt to stabilise the variances. If variances were still heterogeneous a non-parametric test such as a Kruskal-Wallis ANOVA was used.

Organ weights were also analysed conditional on body weight (i.e. analysis of covariance).

Histological data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were 4 premature decedents (one Intermediate dose male, one Low dose female and 2 Intermediate dose females). Three of these animals were killed due to eye damage which occurred during the Week 13 orbital sinus bleed and one animal died during the bleed. None of the deaths were due to treatment with AMPA.

B. CLINICAL OBSERVATIONS

There were no notable clinical signs in either sex.

C. BODY WEIGHT

There were no notable intergroup differences in male animals.

A slight reduction was noted in the overall group mean body weight gain (16 %) of Low dose females (see table below). Comparison with Controls did not reveal any statistical significance at any time interval. Due to the lack of any effects observed in the Intermediate or High dose group females, this reduction was considered not to be related to treatment with AMPA.

Table 6.8.1-11: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted] 1993): Intergroup comparison of group mean body weights and body weight gain

	Mean body weight or body weight gain [g]		
Time point	**Initial body weight**	**Final body weight**	**Total weight gain Time 0 – Week 13**
Dose [mg/kg bw/day]	**Males**		
0	159	512	353
10	157	495 (97%)	338 (96 % of Control)
100	153	488 (95%)	335 (95 % of Control)
1000	155	500 (98%)	345 (98 % of Control)
	Females		
0	115	308	193
10	118	281 (91%)	163 (84 % of Control)
100	118	297 (96%)	179 (93 % of Control)
1000	116	298 (97%)	182 (94 % of Control)

D. FOOD CONSUMPTION

There were no notable intergroup differences for food consumption in either sex.

E. WATER CONSUMPTION

There were no notable intergroup differences for water consumption in either sex.

E. OPHTHALMOSCOPY

All animals examined were considered normal.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

In males, MCH was slightly increased in the Intermediate dose group (5 %, p<0.05). Due to the small magnitude of difference and absence of any effects observed in the High dose group, this change was considered not to be treatment-related.

In females, MCV was slightly reduced in the Low and Intermediate dose groups (both 3 %, p<0.05). However, due to the small magnitude of difference and absence of effects observed in the High dose group or in males, these changes were considered not to be related to treatment. In addition, Neutrophils were increased (66 %, p<0.01) in Low dose group females, however, due the absence of any effects observed in the Intermediate or High dose groups this change was considered not to be related to treatment.

Table 6.8.1.1.6.4-12: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted] 1993): Intergroup comparison of selected group mean haematology parameters (mean ± SD)

Parameter	Dose Groups [mg AMPA/kg bw/day]							
	Males				Females			
	0	**10**	**100**	**1000**	**0**	**10**	**100**	**1000**
MCH [pg]	17.5 ± 0.9 (100%)	17.4 ± 0.4 (99%)	**18.3* ± 0.6** (105%)	17.6 ± 1.5 (101%)	19.2 ± 0.8 (100%)	18.4 ± 0.5 (96%)	18.6 ± 0.5 (97%)	18.8 ± 0.5 (98%)
MCV [fL]	49.0 ± 2.5 (100%)	48.8 ± 1.3 (100%)	50.4 ± 1.6 (103%)	49.4 ± 3.7 (101%)	52.3 ± 1.6 (100%)	**50.7* ± 1.0** (97%)	**50.9* ± 1.1** (97%)	51.9 ± 1.8 (99%)
Neutrophils [× 10^9/L]	2.23 ± 1.00 (100%)	2.78 ± 1.50 (125%)	2.25 ± 0.98 (101%)	3.17 ± 1.39 (142%)	0.98 ± 0.38 (100%)	**1.63** ± 0.42** (166%)	1.06 ± 0.49 (108%)	0.87 ± 0.35 (89%)

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

Clinical chemistry

There were no notable intergroup differences in males.

In females, ALT was increased in the Intermediate dose group (45 %, p<0.05). Due to an absence of any effect observed in the High dose group this increase was considered not to be treatment-related.

Table 6.8.1.1.6.4-13: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted] 1993): Intergroup comparison of selected group mean clinical chemistry parameters (mean ± SD)

Parameter	Dose Groups [mg AMPA/kg bw/day]							
	Males				Females			
	0	**10**	**100**	**1000**	**0**	**10**	**100**	**1000**
ALT [iu/L]	46 ± 10 (100%)	50 ± 7 (109%)	53 ± 27 (115%)	38 ± 4 (83%)	31 ± 6 (100%)	35 ± 7 (113%)	**45* ± 18** (145%)	30 ± 11 (97%)

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

H. TERMINAL STUDIES

Organ weights

In males, thyroid weight was decreased in the Low dose group after correction for final body weight (13 %, p<0.01). Due to an absence of effects observed in the Intermediate or High dose groups, this decrease was considered not to be related to treatment.

In females, ovary weight was reduced in Low and Intermediate dose groups when expressed as an absolute value (14 %, p<0.05 and 17 %, p<0.05, respectively) and after correction for final body weight (14 %, p<0.05 and 17 %, p<0.05, respectively). Uterus weight was increased in Low and Intermediate dose groups when expressed as an absolute value (31 %, p<0.05 and 36 %, p<0.05, respectively) and after correction for final body weight (42 %, p<0.05 and 40 %, p<0.01, respectively). Due to an absence of any effect in the High dose group the above findings were considered not to be related to treatment. Thyroid weight was reduced in the Low dose group when expressed as an absolute value (19 %, p<0.05). Since this change was not seen after correction for final

body weight and due to the lack of effect in the Intermediate or High dose groups, this reduction is considered to be due to chance. Brain weight was slightly reduced (4 %, $p<0.05$) and heart weight was increased (18 %, $p<0.05$) in the High dose group after correction for final body weight. The increase in heart weight was considered to be associated with one animal with myocardial hypertrophy which showed a marked increase in heart weight.
No changes in salivary gland weight were reported among dose groups.

Table 6.8.1.1.6.4-14: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted] 1993): Intergroup comparison of selected absolute and relative mean organ weights

Organ/Tissue [g]	Dose Groups [mg AMPA/kg bw/day]							
	Males				Females			
	0	**10**	**100**	**1000**	**0**	**10**	**100**	**1000**
Thyroids (corrected for body weight; ± SE)	0.023 ± 0.001 (100%)	**0.020** ± 0.001** (87%)	0.022 ± 0.001 (96%)	0.023 ± 0.001 (100%)	0.020 ± 0.001 (100%)	0.018 ± 0.001 (90%)	0.020 ± 0.001 (100%)	0.020 ± 0.001 (100%)
Ovaries (absolute, ± SD)	-	-	-	-	0.105 ± 0.013 (100%)	**0.090* ± 0.012** (86%)	**0.087** ± 0.014** (83%)	0.101 ± 0.014 (96%)
Ovaries (corrected for body weight; ± SE)	-	-	-	-	0.105 ± 0.004 (100%)	**0.090* ± 0.004** (86%)	**0.087** ± 0.005** (83%)	0.103 ± 0.004 (98%)
Uterus (absolute; ± SD)	-	-	-	-	0.55 ± 0.14 (100%)	**0.72* ± 0.23** (131%)	**0.75* ± 0.23** (136%)	0.57 ± 0.11 (104%)
Uterus (corrected for body weight; ± SE)	-	-	-	-	0.54 ± 0.05 (100%)	**0.75* ± 0.06** (139%)	**0.75* ± 0.06** (139%)	0.56 ± 0.06 (104%)
Brain (corrected for body weight; ± SE)	2.11 ± 0.02 (100%)	2.12 ± 0.02 (100%)	2.10 ± 0.02 (100%)	2.11 ± 0.02 (100%)	1.96 ± 0.02 (100%)	1.97 ± 0.03 (101%)	1.97 ± 0.03 (101%)	**1.88* ± 0.03** (96%)
Heart (corrected for body weight; ± SE)	1.46 ± 0.04 (100%)	1.51 ± 0.04 (103%)	1.61 ± 0.04 (110%)	1.54 ± 0.04 (105%)	1.03 ± 0.05 (100%)	1.04 ± 0.05 (101%)	1.07 ± 0.06 (104%)	**1.22** ± 0.05** (118%)

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

Gross pathology
There were no necropsy findings which were considered to be due to treatment with AMPA. All findings were typical of background pathology for this age and strain of rat. For some animals lesions associated with the right eye were noted, however, these were considered to be due to the blood sampling process.

Histopathology
There were no histopathological findings related to treatment. Those findings reported were typical of background pathology for rats of this age and strain.
In order to compare the toxicity of AMPA with the parent compound glyphosate, the histopathological findings of the salivary gland are reported here. No abnormalities were observed in the submaxillary (mandibular) and sublingual salivary gland. In the parotid salivary gland, basophilic hypertrophic foci were observed among all dose groups (see table below) and no treatment-related effect is noted. It is noted, however, that the administration was by gavage in this study.

Table 6.8.1.1.6.4-5: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted]., 1993): Salivary gland findings

Salivary gland (parotid)	Dose Groups [mg AMPA/kg bw/day]							
	Males				Females			
	0	**10**	**100**	**1000**	**0**	**10**	**100**	**1000**
No of animals	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
No abnormalities	5	4	4	3	4	5	2	3

Table 6.8.1.1.6.4-5: 13 Week Toxicity Study in Rats with Administration by Gavage [redacted] 1993): Salivary gland findings

Basophilic hypertrophic focus – grade +/-	4	2	5	5	4	1	8	4
Basophilic hypertrophic focus – grade +	1	4	1	2	2	4	0	3

III. CONCLUSIONS

There were no findings considered to be related to treatment with AMPA.
Brain weight was slightly reduced and heart weight increased in females receiving 1000 mg/kg bw/day. Due to the lack of any correlating findings, these changes were considered not to be reproducible.
In conclusion, dosing of Sprague-Dawley rats orally by gavage for 13 weeks with AMPA produced no effects at 10, 100 or 1000 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study groups of 10 male and 10 female Sprague-Dawley rats were dosed orally for 13 weeks with AMPA at dose levels of 0, 10, 100 or 1000 mg/kg bw/day via gavage. The study was conducted according to OECD 408 (1981) and in compliance with GLP (no attest of the competent authority was provided). Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
However, the study was well conducted, in compliance with GLP and OECD 408 (1981) and thus, can provide useful information for the assessment of the metabolites of glyphosate. The study is therefore considered valid.
There were no findings considered to be related to treatment with AMPA. Brain weight was slightly reduced and heart weight increased in females receiving 1000 mg/kg bw/day. Due to the lack of any correlating findings, these changes were considered not to be reproducible.
In conclusion, dosing of Sprague-Dawley rats orally by gavage for 13 weeks with AMPA produced no effects at 10, 100 or 1000 mg/kg bw/day.
Thus, under the conditions of this study the NOAEL can be set at 1000 mg/kg bw/day.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant and in line with the conclusions in the RAR (2015). No treatment related signs of toxicity were reported in male and female animals up to 1000 mg/kg bw/day. Thus, the NOAEL for males and females is 1000 mg/kg bw/day.

Study 5

Data point	CA 5.8.1/017
Report author	[redacted] *et al.*
Report year	1979
Report title	90-Day Subacute Rat Toxicity Study
Report No	401-050
Document No	M-644184-01-1
Guidelines followed in study	No guideline followed but similar to OECD 408.
Deviations from current test guideline (OECD 408, 2018)	Ophthalmoscopy not performed. Functional observation and/or sensor reactivity assessments were not performed. Blood clotting time/potential not included within haematology parameters. Sodium, potassium, HDL, LDL, urea and creatinine were not evaluated as part of clinical chemistry

	parameters. T4, T3 and TSH were not measured. Vaginal smears for oestrus cycle determination of females not performed at necropsy. Adrenals, epididymides, prostate + seminal vesicles, uterus, thymus, spleen, thyroid and pituitary were not weighed at necropsy. Aorta, cervix, vagina, epididymides, seminal vesicles, coagulation glands, mammary gland and skin were not preserved / fixed. In addition, only one lymph node (mesenteric) was sampled and fixed. Histological assessment of the testes may not be as detailed as current guideline. Identification of the test chemical was limited.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No formal claim of compliance with GLP or specific guidelines since the study was performed pre-GLP. Quality assurance statement is included.
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable but with restrictions (reliable with restrictions).

2. Full summary

Executive summary

The test material (CP 50435) was administered in the diet to Charles River CD® weanling rats at dosage levels of 400, 1200 or 4800 mg/kg bw/day for 90 days. Each dosage level was fed to a group of 20 male and 20 female rats. The control group, containing a like number of rats, was fed only the basal laboratory diet. Water and respective diets were available *ad libitum*. The rats were observed twice daily for mortality and signs of overt toxicity. Detailed observations, individual body weights and food consumption were recorded weekly. Haematological and biochemical determinations and urinalyses were conducted at 45 and 88 days of study on 10 rats from each sex and group. Baseline values of the clinical laboratory tests were obtained from 10 male and 10 female weanling rats sacrificed for this purpose.

No changes considered to be related to the compound were seen in general behaviour, appearance, haematological determinations and urinalyses. A moribund male rat in the control group was sacrificed in week 12. Nine rats from the control and treated groups died following the collection of blood at 45 and 88 days. The male rats at the 4800 mg/kg bw/day dosage level consumed less food and gained less body weight than the control. The difference in mean body weight between the control rats and male and female rats at the 4800 mg/kg bw/day dosage level reached statistical significance.

The lactic dehydrogenase activity (LDH) of the blood from rats at the 4800 mg/kg bw/day dosage level was higher than that of the control rats. For male rats at the 4800 mg/kg bw/day dosage level, the mean LDH activity was 48 and 145 % greater than the control value at 45 and 88 days, respectively. Similarly, for the female rats at the 4800 mg/kg bw/day dosage level, the mean LDH activity was 29 and 171 % greater than the control values at 45 and 88 days, respectively.

All rats which died or were sacrificed in extremis during the course of study or which were sacrificed at scheduled termination were necropsied. A full set of tissues as specified in the protocol was examined microscopically from the control and 4800 mg/kg bw/day groups. Liver, kidneys, heart, urinary bladder and gross lesions were examined microscopically in rats from the 400 and 1200 mg/kg bw/day groups. No compound related gross pathologic lesions were observed at necropsy in any rats from the test compound (CP 50435) treated groups. Statistical variations, of undetermined biological significance, occurred in male and female group mean weights of kidneys, gonads, heart and brain at one or more dosage levels.

Microscopic changes which were considered compound related were limited to the urinary tract of rats from the 4800 and 1200 mg/kg bw/day dose groups. Several rats from the 1200 mg/kg bw/day groups and most rats from the 4800 mg/kg bw/day groups had hyperplasia of the urinary bladder epithelium. Very slight to slight hyperplasia of the epithelium of the kidney pelvis in several rats from the 4800 mg/kg bw/day group also was considered to be compound-related. No compound related urinary tract lesions were observed at the 400 mg/kg bw/day level.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	CP 50435 (Aminomethylphosphonic acid)
Description:	Grainy white crystalline powder/material
Lot/Batch #:	XHI 45 and XHI-136
Purity:	99.96 % (Lot No. XHI-136)
Stability of test compound:	Unknown
2. Vehicle and/ or positive control:	Purina® Laboratory Chow® / none
3. Test animals:	
Species:	Rat
Strain:	Charles River CD®
Source:	[redacted]

Age:	Approx. 4 weeks
Sex:	Male and female
Weight at dosing:	♂ 70 – 93 g; ♀ 67 – 90 g
Acclimation period:	7 days
Diet/Food:	Purina® Laboratory Chow®, *ad libitum* (except during laboratory investigations for Day 45 and 88)
Water:	Tap water, *ad libitum* (except during laboratory investigations for Day 45 and 88)
Housing:	Individual housing in hanging wire-mesh cages
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported Light cycle: 12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

In life dates: 1978-06-27 (start of dosing) to 1978-09-26 (terminal necropsies)

Animal assignment and treatment:
The test material (CP 50435) was fed in the diet at varying concentrations (based upon changes in body weight and food consumption) to provide the dosage levels indicated below:

Table 6.8.1.1.6.5-15: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Treatment Groups

Test group	Dose Group [mg AMPA/kg bw/day]	Number of Rats	
		Males	Females
Control	0	20	20
Low	400	20	20
Intermediate	1200	20	20
High	4800	20	20

The test compound was added to ground Purina® Laboratory Chow® on a weight-to-weight basis. The appropriate amount of the test compound for each dosage level was ground with a portion of the basal diet using a mortar and pestle. This premix was combined with the remaining portion of the basal diet in a twin-shell blender. Initially, as indicated in the protocol, 6000 g of the test diet were prepared weekly and unused diets were discarded at the end of each week. Because of the shortage of test material the total amount of test diets prepared was reduced at week 10. Also, because of the shortage of test material the food consumption periods were adjusted as follows: On day 7 of study week 11, the weight of the food container and remaining food was recorded for each rat and containers and food returned to the animal cage. On day 1 of week 12, the food jars for the male rats were weighed and fresh diets were administered to them. On day 2 of week 12, the food jars for the female rats were weighed and fresh diets administered to them. Because of this, the food consumption data for week 12 was summarised in 2 parts. For the food consumption calculations of the periods ending on day 1 of week 12 for the male treatment groups and on day 2 of week 12 for the female treatment groups the body weights recorded on day 7 of week 11 were used.

Diet Samples
Samples of stored test materials were taken at the beginning of the study and at monthly intervals. Weekly samples of the control feed and each treatment diet was taken on day 1 of feeding. Additional containers of the diets were placed in empty cages at the beginning of each feeding week and sampled at the end of the week. All diet samples were frozen immediately after collection. The samples were shipped to the sponsor at monthly intervals.

Mortality
The animals were observed twice a day 7 days a week for mortality and signs of overt toxicity.

Clinical observations
Detailed observations of each rat were recorded weekly.

Body weight
Individual body weights were recorded weekly.

Food consumption
Individual food consumption was recorded weekly.

Haematology and clinical chemistry and urinalysis
10 male and 10 female rats from each dose level were selected for clinical laboratory investigations at 45 and 88 days of study. The baseline data of the clinical laboratory tests were obtained from 10 male and 10 female weanling rats similarly selected and sacrificed for this purpose. Blood samples were obtained by the orbital sinus technique from fasted rats. The urine was collected while the rats were fasting.

Haematology parameters included: Haematocrit value, haemoglobin concentration, erythrocyte count, leucocyte count (total and differential), platelet count and reticulocyte count.

Biochemistry parameters included: Fasting blood glucose, blood urea nitrogen, total cholesterol, total protein, albumin, globulin (calculated), direct and total bilirubin, serum alkaline phosphatase (SAP), serum lactic dehydrogenase (LDH), serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT).

Urinalysis included description of colour and appearance, determination of volume, specific gravity and pH, qualitative tests for glucose, protein, ketones, bilirubin, urobilinogen and microscopic examination of sediment.

Terminal studies
At completion of the compound feeding period, all surviving rats were sacrificed by carbon dioxide asphyxiation and necropsied. At necropsy, examination was made of the external body surface and orifices and the rat was opened. Contents of the cranium, thorax and abdomen were examined *in situ*, removed, and again examined. Representative tissues of organs from each rat were collected and fixed in buffered neutral 10 % formalin. Rats which died or were sacrificed in extremis during the course of the study were necropsied as above.

The liver, kidneys, testes, heart and brain from each rats sacrificed at termination were weighed at necropsy and ovaries were weighed after fixation.

Haematoxylin and eosin stained paraffin sections of the following tissues from all rats from the control and 4800 mg/kg bw/day groups were prepared by standard histologic methods and examined microscopically: Brain, spinal cord, peripheral nerve (sciatic), eye (optic nerve), pituitary, thyroid (parathyroid), adrenals, trachea, lung/bronchi, heart, spleen, lymph node (mesenteric), thymus, sternum (bone marrow), salivary gland (submandibular), oesophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (colon, caecum), pancreas, liver, kidneys, urinary bladder, testes/ovaries, prostate/uterus, skeletal muscle and any tissues with gross lesions.

Haematoxylin and eosin stained paraffin sections of kidneys, liver, heart, urinary bladder and tissues with gross lesions were prepared and examined from all rats from the 400 and 1200 mg/kg bw/day feeding groups.

Statistics
All statistical analyses compared the treatment groups with the control group, by sex.
Body weights (week 13), food consumption (week 13) haematological, biochemical and urinalysis parameters (days 45 and 88) and absolute and relative organ weights (terminal), were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

II. RESULTS AND DISCUSSION

A. GENERAL BEHAVIOUR, APPEARANCE AND SURVIVAL

One male and five female rats at the 4800 mg/kg/day dosage level were found dead on day 45 following the collection of blood. Similarly, one female in the control group and two female rats in the 1200 mg/kg bw/day

group were found dead after the collection of blood on study day 88. In addition, one male rat in the control group was sacrificed in week 12. Previous observation included soft stools, distended abdomen and morbidity. Survival after 90 days of study was a follows:

Table 6.8.1.1.6.5-16 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted] 1979): Intergroup comparison of group survival

Dose Level [mg/kg bw/day]	Surviving Males / No. Initiated	Surviving Females / No. Initiated
0 (control)	19/20	19/20
400	20/20	20/20
1200	20/20	18/20
4800	19/20	15/20

B. BODY WEIGHT

For both male and female rats at the 4800 mg/kg bw/day dosage level and male rats at the 1200 mg/kg bw/day dosage level, the differences in body weight from that of the control were statistically significant at 13 weeks (see table below).

Table 6.8.1.1.6.5-17: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted] 1979): Intergroup comparison of group mean body weights and body weight gain

Dosage Group [mg/kg bw/day]	Mean body weight or body weight gain [g]		
	Initial body weight	Final body weight	Total weight change [%] Time 0 – Week 13
	Males		
0 (control)	82	480 (100%)	485 (100%)
400	80	480 (100%)	500 (103%)
1200	80	**453*** (94%)	466 (96%)
4800	81	**346**** (72%)	327 (67%)
	Females		
0 (control)	79	264 (100%)	234 (100%)
400	76	266 (101%)	250 (107%)
1200	79	274 (104%)	246 (105%)
4800	79	**252**** (95%)	219 (94%)

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

C. FOOD CONSUMPTION

Treated male rats consumed less food than the control, although when compared with control this was not shown to be statistically significant.

In increasing dosage levels the compound consumption based on food consumption for the male rats was 406, 1230 and 4989 mg/kg bw/day and for the female rats was 388, 1161 and 4625 mg/kg bw/day, respectively.

Table 6.8.1.1.6.5-18 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup Comparison Group Average Food Consumption

Dosage Group	Average Food Consumption [g/rat/day]	
Dose [mg/kg bw/day]	**Males**	**Females**
0 (control)	25.5	17.9
400	25.1 (-1.6)	17.8 (-0.6)
1200	25.2 (-1.2)	19.2 (7.3)
4800	23.5 (-7.8)	18.3 (2.2)

(): % difference from Control

D. CLINICAL LABORATORY TESTS

Haematology
No changes considered to be related to the compound intake were seen in the haematological determinations. For as few specific determinations there were statistically significant differences between the control and some of the treated groups. However, these changes were not dose dependent, usually seen at a single time point or not seen in the opposite sex.

Biochemistry
For the control and treated groups the mean values for the lactic dehydrogenase activity was greater at 45 and 88 days than for the control baseline period and the lactic dehydrogenase activities for the rats at the 4800 mg/kg bw/day dosage level were considerably greater than for the other groups.

The differences between the mean values for these male rats and the control rats were statistically significant and the effect may be regarded as dose related. Similarly, for the male rats at the 1200 mg/kg bw/day dosage level after 88 days the increase in lactic dehydrogenase activity is significant compared to the control and may be dose related.

For the female rats the assessment is complicated by the 25 % decrease in lactic dehydrogenase activity of the control for the determinations at 45 and 88 days. This brings into question the biological significance of the differences between the female control and the female rats at 400 and 1200 mg/kg bw/day for the 88 days determination, especially since the values are not dose related. At the 4800 mg/kg bw/day dosage level the values were definitely greater than the usual range values and the increase is significant. Although the magnitude of the effect at 88 days appeared exaggerated by the lower control values the increase in lactic dehydrogenase activity was real and may be regarded as dose related.

Five male and four female rats at the 4800 mg/kg bw/day dosage level provided blood samples at both 45 and 88 days. For all but one female rat the values at 88 days were greater than those at 45 days.
For a few other specific determinations there were statistically significant differences between the control and some of the treated rats. However, these changes were not dose dependent, usually seen at a single time point or not seen in the opposite sex.

Table 6.8.1.1.6.5-19: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup comparison of selected group mean biochemistry parameters

Parameter	Group Mean Lactic Dehydrogenase Activity [B-B units/mL]							
	Male [mg/kg bw/day]				Female [mg/kg bw/day]			
	0	**400**	**1200**	**4800**	**0**	**400**	**1200**	**4800**
LDH (Baseline)	1645	-	-	-	1361	-	-	-
LDH (45 Days)	1847	1779 (-3.6)	1901 (2.9)	**2738** (48.2)**	1897	1926 (1.5)	1622 (-14.5)	2437 (28.5)
LDH (88 Days)	1893	1584 (-16.3)	**2442* (29.0)**	**4637** (145.0)**	1427	**2075** (45.4)**	**1971** (38.1)**	**3866** (170.9)**

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01; (): % difference from Control

Urinalysis
No changes considered to be related to the compound intake were seen in the urinalysis.

E. TERMINAL STUDIES

Organ weights
Statistically significant variances in group mean organ weights of liver, kidneys, testes / ovaries, heart and brain were noted for males and females. These are most likely secondary to the reduced body weight related at high dose animals. In the absence of compound related morphologic parenchymal changes in these organs, the biological significance of these statistical variations was undetermined.

Table 6.8.1.1.6.5-20: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted] 1979): Intergroup comparison of selected absolute and relative group mean organ weights

Organ/Tissue	Dosage Groups [mg/kg bw/day]							
	Males				Females			
	0	400	1200	4800	0	400	1200	4800
Liver (absolute) [g]	21.21 (100%)	**18.94*** (89%)	**18.08** (85%)	**13.90** (66%)	11.13 (100%)	10.48 (94%)	10.80 (97%)	**9.10**** (82%)
Liver (relative) [%]	4.40 (100%)	4.18 (95%)	4.10 (93%)	**4.04*** (92%)	4.32 (100%)	4.12 (95%)	4.07 (94%)	4.07 (94%)
Kidneys (absolute) [g]	4.26 (100%)	3.94 (92%)	**3.84*** (90%)	**3.27**** (77%)	2.24 (100%)	2.27 (101%)	2.38 (106%)	**2.00**** (89%)
Kidneys (relative) [%]	0.89 (100%)	0.87 (98%)	0.87 (98%)	**0.95*** (107%)	0.87 (100%)	0.89 (102%)	0.90 (103%)	0.93 (107%)
Testes (absolute) [g]	3.60 (100%)	3.60 (100%)	3.54 (98%)	**3.35**** (93%)	-	-	-	-
Testes (relative) [% × 10]	0.76 (100%)	0.80 (105%)	0.81 (107%)	**0.98**** (129%)	-	-	-	-
Ovaries (absolute) [mg]	-	-	-	-	146 (100%)	**114**** (78%)	132 (90%)	130 (89%)
Ovaries [% × 10]	-	-	-	-	0.57 (100%)	**0.45**** (79%)	**0.50*** (88%)	0.58 (102%)
Heart (absolute) [g]	1.67 (100%)	1.57 (94%)	1.55 (93%)	**1.24**** (74%)	1.05 (100%)	0.99 (94%)	1.03 (98%)	**0.88**** (84%)
Heart (relative) [%]	0.35 (100%)	0.35 (100%)	0.35 (100%)	0.36 (103%)	0.41 (100%)	0.39 (95%)	0.39 (95%)	0.39 (95%)
Brain (absolute) [g]	2.17 (100%)	2.13 (98%)	2.12 (98%)	**2.05**** (94%)	1.97 (100%)	1.97 (100%)	**2.04*** (104%)	1.93 (98%)
Brain (relative) [%]	0.45 (100%)	0.47 (104%)	0.49 (109%)	**0.60**** (133%)	0.77 (100%)	0.78 (101%)	0.77 (100%)	**0.86**** (112%)

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

Gross pathology

No gross pathologic lesions which were considered related to test material (CP 50435) feeding were observed at necropsy in any rats from the experimental groups.

Histopathology

Microscopic pathologic lesions which were considered related to feeding of the test material (CP 50435) were limited to the urinary tract of rats from the 1200 and 4800 mg/kg bw/day feeding groups. Several rats from the 1200 mg/kg bw/day feeding groups and most rats from the 4800 mg/kg bw/day group had hyperplasia of the urinary bladder epithelium. Urinary bladders of rats from the 400 mg/kg bw/day group were comparable to those of control group rats. The compound related urinary bladder hyperplasia was more marked in males than in females and in males it was occasionally accompanied by an acute inflammatory infiltrate in the mucosa and submucosa. The lesion, when present, was fairly uniform over most of the circumference of the urinary bladder and did not show papillarity.

Epithelial hyperplasia was noted in the pelvis of the kidney of a few rats in the 4800 mg/kg bw/day group. The hyperplasia had a slight papillarity and was not uniform over the entire epithelial surface. Some hyperplastic epithelial cells contained a hyaline like cytoplasmic inclusion. This lesion was considered compound related. Several other kidneys had areas of pelvic epithelium which appeared to be hyperplastic, but in these the tissue was cut such that the possibility of a tangential section of epithelium could not be eliminated.

Other microscopic findings in these rats were lesions principally of an inflammatory nature, which are commonly seen in untreated rats of this age and strain. Other microscopic lesions in the kidneys from control and experimental group animals were those of an inflammatory nature and were considered early lesions of chronic nephritis, a common spontaneous disease of laboratory rats. These inflammatory lesions were much more common in males than in females. Chronic nephritis had a greater incidence and severity in males.

The fresh haemorrhage noted in the lungs of many rats from the control and 4800 mg/kg bw/day group was considered agonal due to death by carbon dioxide asphyxiation. Other lung lesions were those of a minor inflammatory or degenerative nature which are commonly seen in rats and were not considered significant with respect to treatment. Ample areas of bronchi were present for examination in sections from all rats in the control and 4800 mg/kg bw/day groups. Myocarditis noted in the heart of numerous rats from the control and treated groups was characterised by small focal accumulations of mononuclear inflammatory cells scattered throughout the myocardium. This is a common finding in rats. Changes noted in the hearts of these rats were generally quite minimal and considered typical of the degree of myocarditis ordinarily noted in rats of this age.
Prostate glands of a number of rats from the control and experimental groups had slight to moderate inflammatory changes in the interstitium and within prostate follicles. This lesion is a common finding in male rats. Small focal haemorrhages observed in the thymus of many control and experimental group rats were fresh with intact red blood cells present and were considered agonal. Mild subacute inflammatory changes in the parenchyma and portal areas were seen in the livers of numerous rats from control and experimental groups. These lesions are common findings in rats. A number of rats in the control and experimental groups also had very slight extramedullary haematopoiesis in their livers. The hepatocellular vacuolation noted in livers, primarily of male rats, in the experimental and control groups were characterised by the presence of a single large vacuole which was usually not spherical but had a smooth irregular outline. Many of these vacuoles appeared to contain aluminous fluid. Their incidence was somewhat greater in the control males. They were not characteristic of the vacuoles of fatty degeneration and were not considered biologically significant in this study.

Table 6.8.1.1.6.5-7: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup comparison of selected necropsy observations (unscheduled and terminal sacrifice)

Organ		Dosage Groups [mg/kg bw/day]							
		Males				Females			
		0	400	1200	4800	0	400	1200	4800
Kidneys	hydronephrosis	2	-	-	1	-	-	-	3
	red area / cortico-medullary margin dark red	-	-	-	-	1	1	-	-
Urinary bladder	distended with red fluid	1	-	-	-	-	-	-	-
	calculi	-	-	-	1	-	-	-	-
	thickened	-	-	-	1	-	-	-	-
	mucosal hyperplasia	-	-	2	15	-	-	3	13
	vesiculation, epithelium	-	-	-	-	-	-	1	-
	submucosal lymphoid focus	-	1	-	-	-	-	-	-
	Submucosal/mucosal inflammatory infiltrate	-	-	-	6	-	-	-	-

Assessment and conclusion by applicant:

The study was not conducted according to any guideline or in compliance with GLP (pre-GLP), but following a study design similar to OECD 408. Therefore, the deviations listed above are from the current version of OECD 408 (2018).
However, the study was well conducted and thus, can provide useful information for the assessment of the metabolites of glyphosate. The study is therefore considered valid.
No changes considered to be related to the compound were seen in general behaviour, appearance, haematological determinations or urinalysis.
Male rats at the 4800 mg/kg bw/day group consumed less food and gained less body weight than the control. At week 13, the difference in mean body weight gain between control rats and male and female rats at the 4800 mg/kg bw/day dosage level was statistically significant ($p<0.01$).
Lactic dehydrogenase activity (LDH) for rats at 4800 mg/kg bw/day was greater than controls. In males at 4800 mg/kg bw/day mean LDH activity was 48 and 145 % greater than controls at 45 and 88 days,

respectively. In females at 4800 mg/kg bw/day mean LDH activity was 29 and 171 % greater than control values at 45 and 88 days, respectively.
No compound related gross pathologic lesions were observed at necropsy in any rats from the test compound (CP 50435) treated groups.
Statistical variations of undetermined biological significance occurred in group mean weights of kidneys, gonads, heart and brain at several dosage levels in both sexes. Most statistically significant alterations in organ weights occurred in the high dose groups, though.
Microscopic changes which were considered compound related were limited to the urinary tract of rats from the 4800 and 1200 mg/kg bw/day dose groups where hyperplasia of the urinary bladder epithelium was observed. Additionally, very slight epithelial hyperplasia was noted in the bladders of several rats from the 1200 mg/kg bw/day. Very slight to slight hyperplasia of the epithelium of the kidney pelvis in several rats from the 4800 mg/kg bw/day dose group was also considered compound related.
No compound related urinary tract lesions were observed at the 400 mg/kg bw/day level in either sex.
Based on the incidence of microscopic changes in the mid and high dose groups (urothelial hyperplasia of the urinary bladder), the NOAEL equalled 400 mg/kg bw/day under the conditions of this 90-days oral study with AMPA (CP 50435).

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. Based on the increased urothelial hyperplasia of the urinary bladder in male and female animals the NOAEL is considered 400 mg/kg bw/day.

Study 6

Data point:	CA 5.8.1/018
Report author	[redacted]
Report year	1991
Report title	90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA
Report No	[redacted]-50173
Document No	M-645465-01-1
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Subdivision F, FIFRA, Hazard Evaluation: Human and Domestic Animals, Section 82-1 and also the OECD Toxicity Test Guidelines (ISBN 92-64-12221-4, Section 409; 1981)
Deviations from current test guideline (OECD 409, 1998)	Gall bladder, uterus, thymus, spleen and heart not weighed at necropsy.
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.

2. Full summary
Executive summary
The toxicological potential of AMPA was evaluated in this 90-day sub-chronic toxicity study in outbred beagles. Dosage levels of 10, 30, 100 and 300 mg/kg bw/day were selected for the study.

The test material was administered in capsule form as a single daily dose for 91 or 92 consecutive days. A concurrent control group received empty capsules on a comparable regimen. Each dose group consisted of five males and five females. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily prior to dosing and one hour following dosing. Individual body weights were recorded weekly. Food consumption was recorded daily and reported weekly. Clinical pathologic evaluations were conducted once prior to study initiation and during the 6th and 13th weeks of dosing (weeks 5 and 12). Ocular examinations were conducted prior to study initiation and during the 13th week of dosing. Complete

necropsies were performed on all dogs at study termination. Selected organs were weighed and selected organs were examined microscopically.

Test article administration had no adverse effects at any dose level on survival, general clinical condition or behaviour, body weight, food consumption, clinical pathology parameters or organ weight values.
No treatment related lesions were observed neither at the ocular examinations nor following gross and microscopic examinations of selected tissues.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA (Aminomethylphosphonic acid)
Description:	White solid (powder)
Lot/Batch #:	PIT-9008-2407-T
Purity:	87.8 %
Stability of test compound:	August 1993 (Estimated Expiry). Confirmatory analysis performed by Sponsor from samples of test material at the beginning and end of the study.
2. Vehicle and/ or positive control:	Empty gelatine capsule / none
3. Test animals:	
Species:	Dog
Strain:	Outbred Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 8123 – 11929 g; ♀ 6753 – 10788 g
Acclimation period:	2 weeks
Diet/Food:	Purina® Certified Canine Chow® #5007 (offered 1 – 2 hours each day)
Water:	Tap water, *ad libitum*
Housing:	Individual housing in stainless steel cages
Environmental conditions:	Temperature: 19 – 24 °C (67 – 75 °F) Humidity: 30 – 76 % Air changes: 10 – 15 / hour 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1990-09-05 (start of dosing) to 1990-12-06 (terminal necropsies)

Animal Assignment and Treatment:
In a 90-day oral (capsule) toxicity study groups of 5 outbred beagle dogs per sex received daily doses of 0, 10, 30, 100 or 300 mg/kg bw/day, at approximately the same time each day for 91 or 92 consecutive days. Capsules were administered following the 1 – 2 hours feeding period.

Table 6.8.1.1.6.6-21 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted] 1991): Study design

Group Number	Dose level [mg AMPA/kg bw/day]	Dose level corrected for purity	Number of Animals	
			Males	Females

		[mg/kg bw/day]		
1	0	0	5	5
2	10	8.8	5	5
3	30	26.3	5	5
4	100	87.8	5	5
5	300	263	5	5

The appropriate amount of test material was weighed into tared size gelatine capsules. A sufficient number of capsules were weighed for each entire week of dosing and stored. Individual dosages were adjusted weekly, based on the most recent body weight. One empty gelatine capsule was dispensed daily for each control animal.
Analysis of the test material was conducted by the Sponsor prior to the beginning and at the conclusion of the study. It was demonstrated that AMPA was stable throughout the course of the study. Results were provided by the Sponsor and presented in a separate report (Monsanto Study ML-90-369).

Mortality
All animals were observed twice daily, once each morning and once in the afternoon for mortality and moribundity.

Clinical Observations
All animals were observed daily prior to dosing and one hour following dosing for overt signs of toxicity. Only significant findings were recorded at these observations.

Body Weight
Body weights were recorded weekly for study weeks -1 – 13. Terminal body weights were recorded for each animal on the day of study termination.

Food Consumption
Individual food consumption was recorded daily and the weekly averages reported for the corresponding body weight intervals. Food intake was calculated as mg/kg bw/day.

Ophthalmoscopic Examination
Ocular examinations were conducted prior to study initiation and during the 13th week of dosing. All examinations were conducted using a hand-held slit lamp and an indirect ophthalmoscope, preceded by mydriasis with 1 % topical tropicamide hydrochloride.

Clinical Pathology
Clinical pathology parameters (haematology, serum chemistry and urinalysis) were measured on all dogs once prior to study initiation and during the 6th and 13th week of dosing (study weeks 5 and 12, respectively). Collection of blood and urine samples was conducted prior to feeding and test item administration. Blood samples were taken from the jugular vein. Urine was collected using a catheter except for the following instances where urine for re-evaluation was collected using metabolism cages (15 – 16 hours collection) for male numbers 775 and 793 in the 300 mg/kg bw/day group (weeks 5 and 12, respectively) and female number 817 in the 10 mg/kg bw/day group (week 5). Values for these animals were reported but excluded from group mean calculations.

Haematology
The following parameters were evaluated: Total leukocyte count (white cell), erythrocyte count (red blood cells), haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (Platelet), prothrombin time, activated partial thromboplastin time (APTT), differential white (WBC) count (percent and absolute) for neutrophils (segmented and unsegmented), lymphocytes, monocytes, eosinophils and basophils.

Serum Chemistry
The following parameters were evaluated: Glucose, blood urea nitrogen, creatinine, sodium, potassium, serum aspartate aminotransferase (AST), chloride, calcium, globulin, albumin/globulin ratio, serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALK), total bilirubin (Total Bili), total cholesterol (Cholesterol), total protein, albumin and gamma glutamyltransferase (GGT).

Urinalysis

The following parameters were evaluated: Volume, colour, appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, nitrites, leukocytes and microscopy of sediment.

Terminal Studies

Macroscopic and Microscopic Examination

At study termination, all dogs were anaesthetised by intravenous injection of sodium phenobarbital followed by exsanguination. A complete necropsy was conducted on all animals. Necropsy included, but was not limited to, examination of the external surface, all orifices, the cranial cavity, the external and cut surfaces of the brain and spinal cord, and the thoracic, abdominal and pelvic cavities including viscera. Bone marrow smears were taken at necropsy.

The following organs and tissues were collected and placed in 10 % neutral buffered formalin: Adrenals, aorta, bone with marrow (sternum), brain (forebrain, mid-brain, hindbrain), eyes with optic nerve, femur (with joint), gall bladder, gastrointestinal tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum), heart, kidneys, liver (sections of 2 lobes), all gross lesions, lungs (including bronchi), lymph node (mesenteric and suprapharyngeal), ovaries with mesovarium, pancreas, peripheral nerve (sciatic), pituitary, prostate, salivary gland (submaxillary), skeletal muscle (*vastus medialis*), skin with mammary gland, spinal cord (mid-thoracic, cervical and lumbar), spleen, testes with epididymides, thymus, thyroid gland (both lobes with parathyroid gland), trachea, urinary bladder, uterus with vagina.

After fixation and following staining with haematoxylin and eosin, the above tissues were examined microscopically.

Organ Weights

The following organs were weighed from all animals: Adrenals, brain, kidneys, liver, ovaries, testes with epididymides and thyroid gland (with parathyroids).

Statistics

All means were presented with standard deviations, and the number of sampling units used to calculate the means. All statistical tests were performed by a DEC (Digital Equipment Corporation) computer with appropriate programming. Analysis of body weights, body weight changes, food consumption, clinical laboratory values and absolute and relative organ weights were analysed by a one-way analysis of variance followed by Dunnett's Test. All analyses were conducted using two-tailed tests for minimum significance levels of 5 % and 1 % comparing the treatment groups with vehicle control group by sex.

II. RESULTS AND DISCUSSION

A. MORTALITY

All animals survived to scheduled necropsy.

B. CLINICAL OBSERVATIONS

There were no clinical signs in either sex which could be positively attributed to treatment with the test material.

Numerous clinical signs were observed in both sexes at all dose levels, including controls. These consisted primarily of lacrimation, scabbed, reddened and/or swollen ears, soft stool, diarrhoea, salivation and emesis at the daily examinations prior to dosing. In general, these findings occurred at a similar frequency in the control and treated groups, usually in a single animal, and/or in a non-dose-related manner and were considered incidental.

C. BODY WEIGHT

No treatment related effects were apparent on mean body weights or body weight changes in males and females at dose levels of 10, 30, 100 or 300 mg/kg bw/day.

A mean body weight loss in the 300 mg/kg bw/day female group during weeks 5 – 6 was greater than the loss observed in the control group. However, the severity of loss in females at 300 mg/kg bw/day was due to one female (number 801) and this loss was followed by a substantial body weight gain during weeks 6 – 7. It should be noted that during this time period, red material was found in the faeces of this animal and food intake was notably reduced, for which it was concluded that these effects were transitory and not related to treatment.

The only statistically significant difference in body weight between control and treated groups was an increase in mean body weight gain ($p<0.05$) in females at 300 mg/kg bw/day during weeks 8-9 (see below). No other remarkable differences were observed in body weight data between the control and the 10, 30, 100 and 300 mg/kg bw/day dose groups.

Table 6.8.1.1.6.6-22: 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted] 1991): Intergroup comparison of selected group mean body weights and body weight gain

Dose Group [mg/kg bw/day]	Mean body weight or body weight gain [g]				
	Body weight (Week 0)	Body weight (Week 13)	Body weight gain (week 5 – 6)	Body weight gain (week 6 – 7)	Body weight gain (week 8 – 9)
	Males				
0	9781 ± 1147.8 (100%)	11849 ± 1200.9 (100%)	12 ± 214.3	431 ± 95.2	144 ± 232.4
10	10064 ± 1167.1 (103%)	12724 ± 1187.8 (107%)	-145 ± 133.3	493 ± 128.5	160 ± 199.5
300	9704 ± 830.2 (99%)	11910 ± 1908.7 (101%)	62 ± 250.6	280 ± 120.9	378 ± 89.1
100	9861 ± 1242.9 (101%)	12226 ± 1375.3 (103%)	59 ± 160.5	381 ± 154.9	253 ± 91.2
300	9818 ± 1095.7 (100%)	12297 ± 1573.4 (104%)	-7 ± 198.9	444 ± 79.5	135 ± 183.9
	Females				
0	8606 ± 1084.2 (100%)	10084 ± 1396.9 (100%)	-217 ± 98.0	354 ± 162.1	55 ± 184.4
10	8683 ± 1365.5 (101%)	10108 ± 1067.0 (100%)	-138 ± 126.1	287 ± 44.9	118 ± 145.5
30	8214 ± 726.9 (95%)	9889 ± 491.0 (98%)	-235 ± 77.3	438 ± 175.6	121 ± 54.7
100	8443 ± 1126.7 (98%)	9507 ± 1126.5 (94%)	-93 ± 195.6	378 ± 170.0	51 ± 88.8
300	8360 ± 1190.8 (97%)	9759 ± 1401.6 (97%)	-461 ± 507.7	554 ± 100.8	**291* ± 102.5**

*: Statistically significant from controls, p<0.05

D. FOOD CONSUMPTION

Food consumption was unaffected by treatment at any dose level.
Food consumption values (grams/animal/day and g/kg bw/day) in females at 300 mg/kg bw/day were slightly lower, but not statistically significant, than the control group during weeks 5 – 6. The decreases were due to one female (number 801) in the 300 mg/kg bw/day group, and food consumption in this group during weeks 6 – 7 was comparable to that of the control group. Therefore, the brief reduction in food consumption at this dose level was not considered to be a treatment related effect. A statistically significantly increased (p<0.05) food consumption (g/kg bw/day) was noted in females at 300 mg/kg bw/day during weeks 8 – 9. This was the only statistically significant difference in food consumption between the control and treated groups. All other values in the 10, 30, 100 and 300 mg/kg bw/day groups were comparable with the control group.

Table 6.8.1.1.6.6-23: 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted] 1991): Intergroup comparison of selected food consumption values (means ± SD)

Dose Group [mg/kg bw/day]	Group Mean Food Consumption				
	Food Consumption Week 5 – 6 [mg/dog/day]	Food Consumption Week 5 – 6 [g/kg bw/day]	Food Consumption Week 6 – 7 [mg/dog/day]	Food Consumption Week 6 – 7 [g/kg bw/day]	Food Consumption Week 8 – 9 [g/kg bw/day]
	Males				
0	302 ± 60.2 (100%)	29 ± 7.0 (100%)	343 ± 60.2 (100%)	32 ± 5.7 (100%)	29 ± 5.7 (100%)
10	288 ± 52.2 (95%)	25 ± 2.4 (86%)	353 ± 30.0 (103%)	31 ± 1.2 (97%)	28 ± 1.7 (97%)

300	329 ± 48.7 (109%)	32 ± 6.2 (110%)	344 ± 53.8 (100%)	32 ± 5.2 (100%)	33 ± 5.8 (114%)
100	316 ± 58.9 (105%)	29 ± 5.0 (100%)	353 ± 53.7 (103%)	32 ± 3.3 (100%)	30 ± 3.3 (103%)
300	315 ± 15.0 (104%)	30 ± 3.2 (103%)	357 ± 14.5 (104%)	33 ± 4.1 (103%)	30 ± 4.7 (103%)
	Females				
0	219 ± 35.5 (100%)	24 ± 3.2 (100%)	275 ± 51.9 (100%)	29 ± 3.4 (100%)	26 ± 2.9 (100%)
10	228 ± 29.8 (104%)	25 ± 4.0 (104%)	283 ± 23.8 (103%)	31 ± 4.0 (107%)	29 ± 2.7 (112%)
30	227 ± 12.1 (104%)	26 ± 2.5 (108%)	285 ± 31.0 (104%)	32 ± 3.3 (110%)	30 ± 2.0 (115%)
100	234 ± 18.9 (107%)	27 ± 4.3 (113%)	292 ± 29.8 (106%)	33 ± 4.4 (114%)	28 ± 4.2 (108%)
300	178 ± 80.9 (81%)	21 ± 8.2 (88%)	289 ± 54.3 (105%)	33 ± 3.0 (114%)	**33* ± 4.8** (127%)

*: Statistically significant from controls, $p<0.05$

E. OPHTHALMOSCOPY

No oculopathical lesions indicative of a toxic effect were observed at any dose level.

F. CLINICAL PATHOLOGIC EVALUATION

Haematology

Haematology parameters were unaffected by test article administration in the 10, 30, 100 and 300 mg/kg bw/day group males and females.

Values in the treated groups were generally comparable to those in the control group. The statistically significant differences from the control reported were considered incidental since they occurred at low or mid dose levels without a dose-response relationship or similar effects in the opposite sex.

Serum chemistry

No treatment related effects were apparent on serum chemistry parameters at any dose level.

Urinalysis

No adverse effects on urinalysis parameters were observed at any dose level. None of the differences between the control and treated groups were statistically significant.

G. PATHOLOGY

Macroscopic Examination

At study termination, no treatment related lesions were noted in the 10, 30, 100 or 300 mg/kg bw/day group males and females.

The lesions observed in the treated groups were commonly observed lesions and either occurred at a similar frequency in the control or occurred infrequently (generally in a single animal).

Microscopic Examination

No microscopic lesions indicative of treatment-related effect were apparent at any dose level.

The lesions observed occurred similarly in the control and treated groups or were limited, in general, to single occurrences.

Organ weights

Test article administration did not adversely affect mean absolute organ weights and organ weights relative to final body weight in males and females at dose levels of 10, 30, 100 or 300 mg/kg bw/day.

The only statistically significant differences between the control and treated groups were low ($p<0.05$ or $p<0.01$) mean absolute and relative liver weights in the 100 mg/kg bw/day group females. Decreased liver weights were not similarly observed in females at the higher dose level of 300 mg/kg bw/day and no relationship to treatment was evident. No other remarkable difference in organ weight data were observed between the control group and the 10, 30, 100 and 300 mg/kg bw/day groups.

Table 6.8.1.1.6.6-24: 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted] 1991): Intergroup comparison of selected absolute and relative (to body weight) mean organ weights

Organ / Tissue	Dose Groups [mg/kg bw/day]									
	Males					Females				
	0	**10**	**30**	**100**	**300**	**0**	**10**	**30**	**100**	**300**
Absolute Liver weight [g]	313.74 ± 47.483 (100%)	320.22 ± 48.634 (102%)	314.85 ± 48.712 (100%)	302.04 ± 24.138 (96%)	324.36 ± 44.436 (103%)	294.97 ± 55.697 (100%)	269.93 ± 26.698 (92%)	264.46 ± 14.855 (90%)	**229.85* ± 27.905** (78%)	261.66 ± 34.830 (89%)
Relative Liver weight [g/100 g]	2.658 ± 0.3631 (100%)	2.553 ± 0.1949 (96%)	2.657 ± 0.4170 (100%)	2.470 ± 0.0920 (93%)	2.653 ± 0.2288 (100%)	2.921 ± 0.2044 (100%)	2.728 ± 0.1532 (93%)	2.688 ± 0.1518 (92%)	**2.441** ± 0.1140** (84%)	2.696 ± 0.2353 (92%)

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

III. CONCLUSIONS

Administration of the test material AMPA had no adverse effects at any dose level on the survival, the general clinical condition or behaviour of animals, body weight data, food consumption data, haematology parameters, serum chemistry parameters, urinalysis parameters or organ weight values.
No treatment related lesions were observed at the ophthalmologic examination or at the gross and microscopic examinations of selected tissues.

Based on the results of this study, the highest dose level of 300 mg/kg bw/day administered orally to dogs for 91 or 92 consecutive days was considered to be the NOAEL (No Observed Adverse Effect Level).

Assessment and conclusion by applicant:

The test material AMPA was administered orally via capsule to Beagle dogs at dose levels of nominally 0, 10, 30, 100 and 300 mg/kg bw/day. After adjustment for purity (87.8 %) the dose levels were 0, 8.8, 26.3, 87.8 and 263 mg/kg bw/day.
The study was conducted according to OECD 408 (1981) and in compliance with GLP (no attest of the competent authority was provided). Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409.
However, the study was well conducted, in compliance with GLP and OECD 409 (1981) and thus, can provide useful information for the assessment of the metabolites of glyphosate. The study is therefore considered valid.
Administration of the test material AMPA had no adverse effects at any dose level on the survival, the general clinical condition or behaviour of animals, body weight data, food consumption data, haematology parameters, serum chemistry parameters, urinalysis parameters or organ weight values.
No treatment related lesions were observed at the ophthalmologic examination or at the gross and microscopic examinations of selected tissues.
Based on the results of this study, the highest dose level of nominally 300 mg/kg bw/day (263 mg/kg bw/day after adjustment for purity) administered orally to dogs for 91 or 92 consecutive days was considered to be the NOAEL (No Observed Adverse Effect Level).

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. No compound related signs of toxicity were reported in this study up to 300 mg/kg bw/day. Although there are limitations to the study, it is considered acceptable. After adjustment for the low purity (87.8%) this results in a NOAEL of 263 mg/kg bw/day in males and females. This conclusion is in line with the RAR (2015).

B.6.8.1.1.7. Genotoxicity – *in vitro*

Study 1

1. Information on the study

Data point	CA 5.8.1/019
Report author	[redacted]
Report year	1996
Report title	AMPA: Reverse Mutation Test
Report No	IET 96-0076
Document No	NA
Guidelines followed in study	OECD 471 (1983), OECD 472 (1983), U.S. EPA FIFRA Guidelines, Subdivision F (1991), Japanese MAFF (1985)
Deviations from current test guideline (OECD 471, 1997)	Information on historical control data was not reported. 2-Aminoanthracene was used as sole positive control in the presence of metabolic activation. In the repeat-experiment, no parameter was changed. Both experiments were conducted under the same conditions using the pre-incubation method.
Previous evaluation	Yes, accepted in RAR (2015) as supplementary study
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).

2. Full summary

Executive summary
S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* strain WP2 uvrA were exposed to AMPA, a metabolite of glyphosate, in the presence and absence of metabolic activation (phenobarbital and 5,6 benzoflavone-induced rat liver S9 fraction). Two independent experiments were performed according to the pre-incubation method.
Based on the results of a preliminary range-finding study, in which no cytotoxicity was observed up to 5000 µg/plate, test item concentrations for the reverse mutation assay were selected in the range of 313 – 5000 µg/plate. Solvent (water) and appropriate positive controls were included in all experiments. The bacteria strains were exposed for 48 hours at 37 °C, followed by an inspection of the bacterial background lawn and counting of the revertant colonies for each plate.
Precipitation of the test substance was not reported. In addition, there was no cytotoxicity observed in any test strain up to the highest AMPA concentration, neither in the presence nor absence of S9 mix.
There was no relevant increase in the number of his^+ and trp^+ revertants (exceeding a factor of 2 when compared to solvent controls) observed in any experiment in any strain, neither with nor without metabolic activation.
The number of revertants induced by the solvent control was within expected range for each strain, thus demonstrating an acceptable experimental performance. The positive control compounds induced a marked increase in the number of revertant colonies in all strains, demonstrating the validity of the test system and the functionality of the S9 mix.
Based on the results of the present study and under the conditions of the test, AMPA, a metabolite of glyphosate, is not mutagenic in the Ames pre-incubation test with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: AMPA

Identification:	Not specified
Description:	White powder
Lot/Batch #:	A-960719
Purity:	99.33%
Stability of test compound:	The test item was stable for 1 year at room temperature. The stability of the test item in solvent was not specified.
Solvent used:	Sterile water

2. Control materials:

Negative control: A negative control was not employed in this study.

Solvent control: Sterile water

Solvent/final concentration: 100 µL/plate

Positive controls: Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)	DMSO	0.01
	+S9	2-Aminoanthracene (2-AA)	DMSO	1.0
TA 1535	-S9	Sodium azide (NaN_3)	Water	0.5
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.0
TA 98	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	DMSO	0.1
	+S9	2-Aminoanthracene (2-AA)	DMSO	0.5
TA 1537	-S9	9-Aminoacridine (9-AA)	Water	80.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.0
E. coli strain(s)				
WP2 uvrA	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	DMSO	0.01
	+S9	2-Aminoanthracene (2-AA)	DMSO	10.0

3. Metabolic activation:

S9 mix was purchased from [redacted]; Lot no. RAA-350). The homogenate was produced from the livers of male Sprague-Dawley rats. The animals were about 7 weeks old, weighing 203 - 254 g and received intraperitoneal injections of phenobarbital (30 mg/kg bw on Day 1, each 60 mg/kg bw on Days 2, 3 and 4) and 5,6 benzoflavone (80 mg/kg bw on Day 3). The S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factors.

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADH	4	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				**Bacteria batch checked for**	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

Preliminary cytotoxicity assay:

Pre-incubation assay ± S9 mix:	
Concentrations:	200, 500, 1000, 2000 and 5000 µg/plate
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	A single plate was used per condition.

Mutation assays:

Pre-incubation assay ± S9 mix:	
Concentrations:	313, 625, 1250, 2500 and 5000 µg/plate
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	Triplicates in two independent experiments

B. STUDY DESIGN AND METHODS
Dates of experimental work: 09 Sep – 11 Oct 1996
Finalisation date: 09 Dec 1996

1. Pre-incubation test (PIT):
0.1 mL of test solution, solvent or positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of 100 mM sodium phosphate buffer (in tests without metabolic activation) were pre-incubated with shaking for 20 minutes at 37 °C. Afterwards, 2 mL of molten soft agar (supplemented with 0.5 mM L-histidine and 0.5 mM D-biotin or 0.5 mM L-tryptophan) was added to each test tube. The contents were mixed uniformly and overlaid on a minimal glucose agar plate. Each concentration and the controls were tested in triplicates. After an incubation period of 48 h at 37 °C, bacterial background lawn was inspected and the number of bacterial colonies (his^+ or trp^+ revertants) were counted.

2. Cytotoxicity:
Cytotoxicity was investigated in a preliminary dose-range finding study. Toxicity was detected by a

- reduction in the number of revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments. Results from the preliminary dose-range finding study/cytotoxicity study are included in the study report but are not shown in this study summary.

3. Statistics:
Results were judged without statistical analysis.

4. Acceptance criteria:
The test was valid if:

- The culture of the tester strains, the solution of the test substance and S9 mix were free from contamination or other bacteria.
- A normal number of spontaneous revertant colonies was observed for the solvent control.
- An at least 3-fold increase above the solvent control in the mean number of revertants was observed in the positive control.

5. Evaluation criteria:
Results were judged positive without statistical analysis when the following criteria were met:

- A two-fold or greater increase above solvent control in the mean number of revertants was observed.
- This increase in the number of revertants was accompanied by a dose-response relationship.
- This increase in the number of revertants was reproducible.

Reproducibility of results was confirmed by two independent experiments.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study.

B. CYTOTOXICITY
In the preliminary cytotoxicity test, as well as in the main gene mutation assay, the test substance did not show any cytotoxicity in any strain up to the highest dose of 5000 µg/plate, neither in the presence, nor in the absence of metabolic activation.

C. SOLUBILITY
Precipitation of the test substance was not reported.

D. MUTATION ASSAY

A relevant increase (≥ 2-fold when compared to corresponding solvent controls) in the number of his^+ or trp^+ revertants was not observed in any experiment at any tested concentration neither in the presence nor absence of metabolic activation.

The number of revertants induced by the solvent control was within the expected range for each strain, thus demonstrating an acceptable experimental performance.

The positive control compounds induced a marked increase in the number of revertant colonies in all strains, demonstrating the validity of the test system and the functionality of the S9 mix.

Table B.6.8.1.1.7.1-1: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1996), first experiment

Experiment 1: Pre-incubation test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	101	105	11	10	17	19	18	30	4	10
± *SD*	± *17*	± *11*	± *1*	± *4*	± *4*	± *1*	± *3*	± *5*	± *3*	± *1*
Test item [µg/plate]										
313 mean	84	105	10	12	17	16	14	28	5	9
± *SD*	± *7*	± *5*	± *5*	± *2*	± *6*	± *4*	± *2*	± *5*	± *3*	± *3*
625 mean	91	92	8	6	17	16	16	28	4	13
± *SD*	± *14*	± *6*	± *6*	± *1*	± *7*	± *1*	± *4*	± *7*	± *2*	± *2*
1250 mean	97	90	8	6	14	16	13	25	4	11
± *SD*	± *17*	± *3*	± *3*	± *1*	± *2*	± *2*	± *2*	± *7*	± *2*	± *3*
2500 mean	91	83	9	9	15	20	15	25	6	10
± *SD*	± *7*	± *9*	± *1*	± *4*	± *8*	± *4*	± *2*	± *8*	± *2*	± *3*
5000 mean	100	93	7	10	16	24	16	32	3	7
± *SD*	± *4*	± *10*	± *4*	± *4*	± *4*	± *6*	± *5*	± *10*	± *1*	± *1*
Positive control										
[§] mean	619	529	619	184	160	384	667	407	710	94
± *SD*	± *57*	± *33*	± *45*	± *5*	± *22*	± *20*	± *60*	± *11*	± *73*	± *2*

[§] = information on respective positive control is reported in Material and Method section A.2

Table B.6.8.1.1.7.1-2: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1996), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	120	95	9	8	15	17	18	28	3	7
± *SD*	± *3*	± *3*	± *3*	± *2*	± *3*	± *3*	± *4*	± *5*	± *2*	± *2*
Test item [µg/plate]										
313 mean	136	112	4	8	18	17	14	21	4	10

Table B.6.8.1.1.7.1-2: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1996), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
± *SD*	± *9*	± *14*	± *1*	± *3*	± *3*	± *4*	± *4*	± *6*	± *3*	± *5*
625 mean	124	84	5	7	16	16	13	21	3	7
± *SD*	± *16*	± *5*	± *2*	± *4*	± *3*	± *5*	± *3*	± *5*	± *2*	± *3*
1250 mean	107	106	6	7	12	17	15	28	3	7
± *SD*	± *11*	± *8*	± *4*	± *2*	± *4*	± *4*	± *2*	± *9*	± *3*	± *1*
2500 mean	95	97	9	11	12	16	16	21	4	6
± *SD*	± *6*	± *4*	± *4*	± *3*	± *3*	± *2*	± *6*	± *1*	± *0*	± *1*
5000 mean	117	115	7	9	20	22	13	22	3	6
± *SD*	± *2*	± *12*	± *3*	± *5*	± *5*	± *2*	± *2*	± *3*	± *2*	± *5*
Positive control										
[§] mean	668	584	696	169	182	461	650	334	698	82
± *SD*	± *27*	± *56*	± *20*	± *28*	± *16*	± *8*	± *8*	± *14*	± *53*	± *4*

[§] = information on respective positive control is reported in Material and Method section A.2

Study conclusion:
According to the results of the present study, the test item is not mutagenic in the Ames pre-incubation test with and without metabolic activation under the experimental conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In the present study, AMPA was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) in the Ames pre-incubation test with and without metabolic activation.
The study was conducted in accordance with OECD guideline 471 and 472 (1983) and in compliance with GLP. When compared with the current OECD guideline 471 (1997), deviations of minor degree were observed. The study is considered supplementary because two tests were performed under identical conditions (using the pre-incubation method only and a plate-incorporation test was apparently not performed).

Assessment and conclusion by RMS:
It is agreed with the applicant that, in the present study, AMPA was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) in the Ames pre-incubation test with and without metabolic activation. Due to the noted deviations, however, the study is considered supportive only.
The study was considered supportive in the previous evaluation (RAR, 2015).

Study 2

1. Information on the study

Data point	CA 5.8.1/020
Report author	[redacted]
Report year	1993
Report title	Mutagenicity test: Ames Salmonella Test with AMPA, batch 286-JRJ-73-4
Report No	13269
Document No	145-GLY

Guidelines followed in study	OECD 471 (1983), US EPA FIFRA 84-2
Deviations from current test guideline (OECD 471, 1997)	The test was conducted in 4 valid strains only. Strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of oxidising mutagens, cross-linking mutagens and hydrazines were not included. In addition, 2-aminoanthracene was used as sole positive control substance in the presence of S9 mix. Historical control data were not provided. Acceptance criteria were not defined and evaluation criteria did not consider historical control data.
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).

2. Full summary

Executive summary

AMPA, metabolite of glyphosate, was investigated for its potential to cause gene mutation in bacteria (Ames test). *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 were exposed to the test item, solvent (distilled water) and appropriate positive controls in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Dose levels were selected based on the results of a preliminary toxicity test in strain TA 98, in which no cytotoxicity was observed up to the highest tested concentration of 5000 µg/plate.
In the main experiment, two independent experiments were performed, using the plate incorporation method (standard plate test, first experiment) and the pre-incubation method (second experiment) and test item concentrations in the range of 310 – 5000 µg/plate.
Precipitation of the test item was not reported. In both experiments, there was no cytotoxicity, evident as a reduction in the number of revertant colonies or a diminution of the bacterial background lawn observed up to the highest concentration of 5000 µg/plate, neither in the presence, nor in the absence of S9 mix.
In addition, there was no statistically significant and biologically relevant increase in the number of revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of metabolic activation. A single statistically significant increase in the number of revertant colonies was observed for strain TA 1535 at 630 µg/plate in the absence of S9 mix, but as the increase was only of marginal magnitude and without any dose response relationship, the effect was considered to be incidental.
The number of revertant colonies induced by the negative and the positive controls were within the expected range, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.
Under the experimental conditions chosen, AMPA, metabolite of glyphosate, is not mutagenic in the Ames standard plate and pre-incubation test with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminomethyl phosphonic acid (AMPA, metabolite of glyphosate)

Identification:	AMPA
Description:	White powder
Lot/Batch number:	286-JRJ-73-4
Purity:	99.2%
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark) was guaranteed for at least 3 years. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	Distilled water

2. Control materials:

Negative control:	A negative control was not employed in this study.
Solvent (vehicle) control:	Distilled water
Solvent (vehicle)/final concentration:	0.1 mL per plate.

Positive controls: Please refer to table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
S. typhimurium strains			
TA 100	-S9	Sodium azide	1.0
	+S9	2-Aminoanthracene	1.5
TA 1535	-S9	Sodium azide	1.0
	+S9	2-Aminoanthracene	1.5
TA 98	-S9	2-Nitrofluorene	1.0
	+S9	2-Aminoanthracene	1.5
TA 1537	-S9	2-Nitrofluorene	1.0
	+S9	2-Aminoanthracene	1.5

3. Metabolic activation:

S9 mix was obtained from the livers of SPF Wistar rats (Mol:WIST) weighing approx. 200 g. The animals received a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bw. The livers were prepared 5 days after treatment. The S9 mix was thawed prior to each experiment and co-factors were immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	5	% (v/v)

4. Test organisms:

Tester strains				**Bacteria batch checked for**	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*62 - 5000 µg/plate*
Tester strains:	TA98
Replicates:	*Not specified*

(b) Mutation assays:

Plate incorporation test ± S9 mix:	
Concentrations:	*310, 630, 1300, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98 and TA 100
Replicates:	*Triplicates*
Pre-incubation test ± S9 mix:	
Concentrations:	*310, 630, 1300, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98 and TA 100
Replicates:	*Triplicates*

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 20 Oct – 06 Nov 1992
Finalisation date: 18 Feb 1993

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution, vehicle or positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation only) were added to 2 mL of molten top agar (supplemented with 0.05 mM histidine and 0.05 mM biotin). After whirl mixing, the mixture was spread on a Vogel-Bonner agar plate and incubated for 48 – 72 hours at 37 °C. Each concentration and the controls were tested in triplicates. Following incubation, the bacterial background lawn was examined and the number of his^+ revertants colonies were counted.

3. Pre-incubation test (PIT):
0.1 mL of test solution, vehicle or positive control, 0.1 mL bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation only) were mixed and pre-incubated in a test tube for 30 minutes at 37 °C under gentle shaking. After pre-incubation, 2.0 mL of top agar was added, the mixture was whirl mixed and spread on a Vogel-Bonner agar plate. After an incubation period of 48 – 72 hours at 37 °C the bacterial background lawn was examined and the number of his^+ revertants colonies were counted.

4. Cytotoxicity
Toxicity was detected by a

- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. Statistics
Statistical analysis of the negative control versus test data was performed using the Analysis of Variance method.

6. Acceptance criteria
Acceptance criteria were not specified in the study report.

7. Evaluation criteria
A test item was considered positive (mutagenic) in the assay if the following criteria were met:

- There was a statistically significant and dose related increase in the level of revertants on the test plates as compared to the control plates.
- The number of revertants at the dose level were the highest effect was found should be more than twice the concurrent spontaneous level.

Sporadic occurring statistically significant increases in revertants which were not dose related (i.e. occurring at the lower dose level when there was no increase at higher non-toxic doses) were considered incidental and not relevant for the evaluation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study.

B. CYTOTOXICITY
In the preliminary toxicity test in strain TA 98, cytotoxicity measured as a colony count reduction was not observed at any dose level, neither in the presence nor absence of S9 mix.
In the main mutagenicity assay there was no cytotoxicity observed for any tester strain up to the highest tested concentration of 5000 µg/plate, neither with nor without S9 mix. In addition, there was no depression of the background growth observed in any tester strain at any concentration, neither in the presence nor absence of S9 mix.

C. SOLUBILITY
Precipitation was not reported in the study report.

D. MUTATION ASSAY
There was no statistically significant and biologically relevant increase in the number of his^+ revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of S9 mix. A single statistically significant increase in the number of revertant colonies was observed for TA 1535 at

630 µg/plate in the absence of S9 mix, but as the increase was only of marginal magnitude and without any dose response relationship, the observation was considered to be incidental.
The number of revertant colonies induced by the negative and the positive controls were within the expected range, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.

Table B.6.8.1.1.7.2-1: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1993), first experiment

Experiment 1: Standard plate test (SPT)								
Strain	**TA 100**		**TA 98**		**TA 1537**		**TA 1535**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control (distilled water)								
mean	130.7	126.7	28.7	33.0	8.3	13.0	16.3	14.3
± *SD*	± *26.5*	± *6.8*	± *17.0*	± *6.0*	± *5.1*	± *4.4*	± *4.0*	± *3.1*
Test item [µg/plate]								
310 mean	119.0	127.3	20.0	28.3	6.7	10.7	16.7	13.7
± *SD*	± *15.9*	± *7.6*	± *5.0*	± *2.5*	± *3.8*	± *3.1*	± *4.2*	± *2.3*
630 mean	116.3	117.0	23.0	34.3	6.3	11.3	**22.7***	15.7
± *SD*	± *14.6*	± *18.5*	± *4.6*	± *4.7*	± *5.1*	± *2.9*	***± 2.1***	± *2.5*
1300 mean	115.7	118.3	23.3	34.7	6.7	10.3	15.3	11.0
± *SD*	± *12.7*	± *19.6*	± *4.2*	± *6.5*	± *3.2*	± *3.5*	± *4.6*	± *3.5*
2500 mean	118.3	116.7	23.3	35.7	6.0	10.7	19.0	18.0
± *SD*	± *9.8*	± *2.5*	± *1.5*	± *3.8*	± *1.0*	± *5.5*	± *1.7*	± *7.0*
5000 mean	101.0	113.7	29.3	34.7	8.0	10.0	13.7	15.7
± *SD*	± *1.7*	± *2.5*	± *15.5*	± *7.8*	± *1.7*	± *4.4*	± *3.2*	± *3.8*
Positive control								
[§] mean	**660.0****	**1706.7****	**320.0****	**1203.3****	**143.3****	**150.0****	**473.3****	**340.0****
± *SD*	***± 165.2***	***± 194.3***	***± 20.0***	***± 119.3***	***± 15.3***	***± 17.3***	***± 68.1***	***± 45.8***

[§] = information on respective positive control is reported in Material and Method section A.2
[*:] statistically significant at the 5% level (analysis of variance)
[**:] statistically significant at 1 % level (analysis of variance)

Table B.6.8.1.1.7.2-2: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1993), second experiment

Experiment 2: Pre-incubation test (PIT)								
Strain	**TA100**		**TA 98**		**TA 1537**		**TA 1535**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control (distilled water)								
mean	143.0	143.0	22.7	26.3	15.0	11.0	14.0	20.7
± *SD*	± *21.3*	± *13.1*	± *4.5*	± *2.1*	± *1.0*	± *4.6*	± *1.0*	± *4.9*
Test item [µg/plate]								
310 mean	138.3	134.7	22.0	28.3	14.3	14.7	12.7	13.3
± *SD*	± *16.2*	± *15.0*	± *4.6*	± *2.3*	± *3.2*	± *2.5*	± *3.1*	± *3.8*
630 mean	129.0	125.3	21.7	26.3	17.7	11.3	15.7	14.7
± *SD*	± *15.0*	± *2.5*	± *5.5*	± *4.0*	± *1.5*	± *2.1*	± *2.1*	± *2.3*
1300 mean	134.0	133.3	21.3	32.7	15.3	16.0	13.3	12.7
± *SD*	± *26.0*	± *14.2*	± *2.3*	± *3.1*	± *4.0*	± *1.7*	± *0.6*	± *1.5*

Table B.6.8.1.1.7.2-2: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1993), second experiment

Experiment 2: Pre-incubation test (PIT)								
Strain	**TA100**		**TA 98**		**TA 1537**		**TA 1535**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
2500 mean	125.0	122.7	26.7	29.3	16.7	15.7	14.3	16.0
± *SD*	± *13.0*	± *7.0*	± *1.2*	± *4.5*	± *3.5*	± *4.0*	± *2.5*	± *5.6*
5000 mean	109.0	140.7	24.3	26.7	10.3	11.3	13.7	16.0
± *SD*	± *4.4*	± *12.5*	± *0.6*	± *6.0*	± *4.0*	± *2.1*	± *3.5*	± *4.4*
Positive control								
§ mean	**700.0****	**910.0****	**266.7****	**893.3****	**123.3****	**310.0****	**500.0****	**210.0****
± *SD*	***± 219.3***	***± 157.2***	***± 20.8***	***± 136.5***	***± 5.8***	***± 45.8***	***± 45.8***	***± 17.3***

§ = information on respective positive control is reported in Material and Method section I.A.2

**: statistically significant at 1 % level (analysis of variance)

III. CONCLUSIONS

Based on the experimental findings, AMPA, metabolite of glyphosate was negative for gene mutation in bacteria in the Ames standard plate test and the pre-incubation test in the presence and absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this Ames test, AMPA was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537) with and without metabolic activation.

The study was conducted in accordance with OECD guideline 471 (1983) and compliant with GLP. It is considered to provide supporting information, as both experiments were conducted using only 4 valid tester strains. Strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of cross-linking mutagens were not included. Further deviations from the currently valid OECD guideline 471 (1997) were of minor degree and considered to not compromise the validity of the study.

Assessment and conclusion by RMS:

It is agreed with the applicant that, in the present study, AMPA was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537) in the Ames standard plate test and the pre-incubation test with and without metabolic activation. Since only four strains were tested, and a strain able to detect certain oxidising mutagens, cross-linking agents and hydrazines was not included, the study is considered acceptable but with restrictions (reliable with restrictions).

The study was considered acceptable in the previous evaluation (RAR, 2015).

Study 3

1. Information on the study

Data point	CA 5.8.1/021
Report author	[redacted]
Report year	1988
Report title	Aminomethyl Phosphonic Acid - An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli*
Report No	CTL/P/2206
Document No	YV2281
Guidelines followed in study	OECD 471/472 (1983); OECD 472 (1983)
Deviations from current test guideline (OECD 471 , 1997)	2-Aminoanthracene was used as sole positive control substance in the presence of S9 mix for all strains. Historical control data were not provided. Evaluation of cytotoxicity and precipitation were not reported, but concentrations were tested up to limit doses. No confirmation in the report of bacterial cell density at the

	time of treatment. Acceptance and evaluation criteria specified in the study report were not fully in accordance with those specified in OECD guideline 471 (1997).
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).

2. Full summary

Executive summary

S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, and *E. coli* strain WP2 uvrA pKM101 were exposed to aminomethyl phosphonic acid in the presence and absence of metabolic activation (Aroclor 1254 -induced rat liver S9 fraction). For each strain, two independent experiments were conducted according to the standard plate test (plate-incorporation method) at test item concentrations in the range of 1.8 – 5000 µg/plate. Solvent (deionized water) and appropriate positive controls were included in each experiment. After 64 – 68 hours of incubation, the bacterial background lawn was inspected and the number of revertant colonies was examined.

Evaluation of precipitation and cytotoxicity was not provided in the study report. Cytotoxicity was evaluated retrospectively by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding solvent control. Cytotoxicity was observed in the first experiment for strain TA 1538 at 1000 µg/plate in the absence of S9 mix and in the second experiment for strain TA 1537 at 5000 µg/plate in the presence of S9 mix.

There was no statistically significant, reproducible increase in in the number of his^+ and trp^+ revertant colony numbers in any of the six tester strains at any dose level, neither in the presence nor absence of metabolic activation. Statistical significance in the mean number of revertant colonies was observed for all strains at single test item concentrations in the presence or absence of metabolic activation. However, none of the observations was consistent in independent experiments or showed a dose-response relationship. For strain TA 1537 in the first experiment, the increase in the mean number of revertant colonies was up to 2-fold over control conditions (200 µg/plate) in the absence of S9 mix and up to 1.9-fold over control conditions (8 and 40 µg/plate) in the presence of S9 mix. In both cases, the responses were only of limited dose-response relationship and of limited statistical significance ($p > 0.01$). Besides, the effects were not reproducible in two further experiments with this strain. Considering all available data of the present study, there was no consistent, statistically significant or biologically relevant increase in the mean number of his^+ or trp^+ revertant colonies observed for any tester strain up to the highest concentration of 5000 µg/plate.

The positive control compounds showed the expected results of markedly increased numbers of revertant colonies, indicating the functionality of the S9 mix and demonstrating the validity of the test system.

Based on the results and under the experimental conditions of the present study, there is no indication for mutagenicity in the Ames standard plate test in the presence and absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminomethyl phosphonic acid

Identification:	Y06384/001/001
Description:	White solid
Lot/Batch #:	48F-3893
Purity:	> 99 %
Stability of test compound:	Not specified.
Solvent (vehicle) used:	Sterile deionised water

2. Control materials:

Negative control:	Not specified.
Solvent (vehicle) control:	Sterile deionised water
Solvent (vehicle)/ final concentration:	100 µL per plate

Positive controls: Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	1.0, 2.0 and 5.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.2, 0.5 and 1.0
TA 1535	-S9	N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	1.0, 2.0 and 5.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.5, 1.0 and 2.0
TA 98	-S9	Daunomycin Hydrochloride (DR)	DMSO	0.2, 0.5 and 1.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.2, 0.5 and 1.0
TA 1537	-S9	Acridine Mutagen ICR191	DMSO	0.5, 1.0 and 2.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.5, 1.0 and 2.0
TA 1538	-S9	4-Nitro-o-phenylenediamine (4NOPD)	DMSO	1.0, 2.0 and 5.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.2, 0.5 and 1.0
E. coli strains				
WP2 uvrA	-S9	N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	0.5, 1.0 and 2.0
	+S9	2-Aminoanthracene (2AA)	DMSO	1.0, 2.0 and 5.0

3. Metabolic activation:

S9 mix was obtained from the livers of male Sprague-Dawley rats, that received a single dose of 500 mg/kg bw Aroclor 1254 by intraperitoneal injection. The treated animals were kept on a normal diet for three days, starved of food but not water on the fourth day and the livers were prepared on the fifth day. A 25 % (w/v) homogenate fraction was prepared and aliquots were frozen. The S9 mix was thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Na_2HPO_4 buffer	150	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP, Na salt	4	mM
$MgCl_2$	8	mM
S9	25	% (v/v)

4. Test organisms:

Tester strains				**Bacteria batch checked for**	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA pKM101	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 P		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538	✓			Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

Plate incorporation test ± S9 mix:	
Concentrations:	1.6, 8.0, 40.0, 200, 1000 and 5000 µg/plate
Tester strains:	TA 1535, TA 1537, TA 1538, TA 98, TA 100 and WP2 uvrA
Replicates:	Triplicates

B. STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 10 – 23 May 1988
   Finalisation date: 21 Sep 1988

2. Standard plate test (plate-incorporation test, SPT):

An aliquot of 0.1 mL test solution or solvent/ positive control, an aliquot of 0.1 mL bacterial overnight culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of sucrose-tris-EDTA buffer (in tests without metabolic activation) were added to 2 mL of molten top agar (supplemented with 0.5 mM histidine/ 0.5 mM biotin stock solution (10 mL solution : 100 mL agar) or 10 mL 0.5 mM tryptophan solution per 100 mL agar). The resulting mixture was poured rapidly onto Vogel Bonner agar plates. The plates were allowed to gel and incubated inverted at 37 °C for 64 - 68 h in the dark. Afterwards, the plates were checked for microbial contamination, the background bacterial lawn was examined and the number of the bacterial colonies (his^+ or trp^+ revertants) was counted.

All strains were tested in two independent experiments and a third experiment was performed for tester strain TA1537 in view of the inconsistent results. In each experiment, all test item concentrations and the controls were tested in triplicates.

3. Cytotoxicity

Evaluation of cytotoxicity was not further specified in the study report. Thus, retrospectively, cytotoxicity was evaluated by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding solvent control. Cytotoxicity was considered evident when the mean number of revertant colonies induced by the test item concentration was ≤ 0.5 fold of the mean number of spontaneous revertant colonies induced by the solvent control.

4. Statistics

An assessment of statistical significance was carried out using a one-tailed Student's t-test. The corresponding probability for each dose level was derived by computer using the appropriate degrees of freedom. Values of $p < 0.01$ were treated as significant with values of $0.01 \leq p < 0.05$ being indicative of a possible effect.

5. Acceptance criteria

The test was valid if;

- The concurrent solvent control data were acceptable.
- The positive control data showed unequivocal positive responses.
- At least the lowest test item dose showed no evidence of toxicity, and at least three test item doses showed no significant overt toxicity.

Failure of one or more tester strain / S9 combinations did not invalidate the data for the remainder of a concurrent experiment.

6. Evaluation criteria

A positive response in an individual experiment was achieved when one or both of the following criteria were met:

- A statistically significant dose-related increase in the mean number of revertant colonies was obtained.
- A two-fold or greater increase of statistical significance in the mean number of revertant colonies was observed at one or more concentrations.

For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain / S9 combination, then the observed effects must be consistently reproducible.

A negative response in an individual experiment was achieved when

- There was no statistically significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance.
- In the absence of any such dose response, no increase in colony numbers was observed at any test concentration, which exceeded 2x the concurrent solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study.

B. CYTOTOXICITY

Cytotoxicity, evident as a reduced number of revertant colonies was observed in the first experiment for strain TA 1538 at 1000 µg/plate in the absence of S9 mix and in the second experiment for strain TA 1537 at 5000 µg/plate in the presence of S9 mix.

C. SOLUBILITY

There was no precipitation of the test substance reported.

D. MUTATION ASSAY

Although a statistically significant increase in the mean number of revertant colonies was observed in the first experiment in strains TA 1535, TA 1537 and WP2 uvrA in the absence of S9 mix and in strains TA 1535, TA 1537, TA 1538, TA 98 and WP2 uvrA in the presence of S9 mix, as well as in the second experiment in strain TA 1535 in the absence of S9 mix and in strains TA 100 and WP2 uvrA in the presence of S9 mix, none of these observations showed a dose-response relationship. In addition, the observations were not reproducible in independent experiments. For strain TA 1537 in the first experiment, the increase in the mean number of revertant colonies was up to 2-fold over control conditions (200 µg/plate) in the absence of S9 mix and up to 1.9-fold over control conditions (8 and 40 µg/plate) in the presence of S9 mix. In both cases, the responses were only of limited dose-response relationship and of limited statistical significance ($p > 0.01$). Therefore, for strain TA 1537 a third experiment was conducted under the same experimental conditions. In the latter two experiments, no significant increases in colony numbers were observed for TA 1537 either with or without S9 mix. Considering all available data of the present study, there was no consistent, statistically significant or biologically relevant increase in the mean number of his^+ or trp^+ revertant colonies observed for any tester strain up to the highest concentration of 5000 µg/plate.

The positive control compounds showed the expected results of markedly increased numbers of revertant colonies, indicating the functionality of the S9 mix and demonstrating the validity of the test system.

Table B.6.8.1.1.7.3-1: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 1988), first experiment, plate-incorporation test

Experiment 1: Standard plate test (SPT, plate-incorporation method)												
Strain	**TA 1535**		**TA 1537**		**TA 1538**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control												
Water mean	13.0	10.2	7.8	5.8	13.0	14.4	22.8	17.8	101.0	95.3	126.8	105.4
± *SD*	*± 4.0*	*± 2.3*	*± 5.7*	*± 2.8*	*± 2.0*	*± 4.2*	*± 5.3*	*± 6.0*	*± 8.5*	*± 9.9*	*± 24.0*	*± 12.3*
Test item [µg/plate]												
1.6 mean	**20.0***	15.3	10.3	9.7	13.3	18.7	20.7	26.0	94.0	98.3	**157.0***	111.3
± *SD*	***± 2.0***	*± 6.1*	*± 5.1*	*± 4.2*	*± 1.2*	*± 1.2*	*± 1.5*	*± 6.0*	*± 8.7*	*± 6.8*	***± 12.8***	*± 7.4*
8.0 mean	**20.7***	17.0*	14.7	11.3	14.3	24.3*	22.3	20.7	101.3	98.3	128.7	107.7
± *SD*	*± 1.2*	*± 4.6*	*± 4.9*	*± 6.4*	*± 2.5*	*± 6.7*	*± 10.8*	*± 5.0*	*± 6.7*	*± 10.6*	*± 7.5*	*± 10.4*
40 mean	14.3	13.3*	10.3	**11.0***	9.0	18.0	22.7	25.3*	95.7	89.0	**161.0***	99.3
± *SD*	*± 4.2*	*± 0.6*	*± 2.1*	***± 1.0***	*± 3.6*	*± 1.4*	*± 6.7*	*± 1.2*	*7.2*	*± 11.1*	***± 13.5***	*± 10.6*
200 mean	**18.7***	**13.7***	**15.3***	**10.3***	9 3	16.0	25.3	**25.7***	94.3	102.0	138.0	119.7
± *SD*	***± 0.6***	***± 2.5***	***± 2.3***	*± 2.3*	*± 4.7*	*± 2.6*	*± 10.0*	***± 2.1***	*± 6.7*	*± 12.5*	*± 27.9*	*± 11.2*
1000 mean	**18.3***	**15.7***	12.3	6.7	6.7	14.7	22.0	20.7	92.0	97.0	149.3	**135.7***
± SD	**± 0.6**	**± 5.5**	± 3.5	± 2.9	± 4.2	± 2.3	± 8.7	± 3.8	± 3.6	± 5.3	± 16.3	**± 14.5**
5000 mean	15.3	11.3	12.3	**9.3***	10.0	15.3	21.3	23.0	89.7	96.0	150.0	104.0
± *SD*	*± 4.2*	*± 1.5*	*± 2.9*	***± 1.5***	*± 6.1*	*± 2.1*	*± 3.2*	*± 4.6*	*± 6.5*	*± 3.6*	*± 19.1*	*± 19.1*
Positive control												
§ 1. mean	**34.0****	**38.5****	**82.0****	**20.5****	**80.0****	**34.0****	**119.0****	**31.0***	**179.5****	**143.0****	**217.0****	**127.5***
± *SD*	***± 5.7***	***± 4.9***	***± 7.1***	***± 0.7***	***± 11.3***	***± 2.8***	***± 28.3***	***± 2.8***	***± 14.8***	***± 5.7***	***± 8.5***	***± 3.5***
§ 2. mean	**2747 5****	**66.0****	**115.5****	**28.0****	**153.0****	**161.0****	**518.5****	**111.5****	**1003 5****	**327.0****	**944.5****	**139.5***
± *SD*	***± 204.4***	***± 8.5***	***± 2.1***	***± 12.7***	***± 8.5***	***± 15.6***	***± 17.7***	***± 4.9***	***± 219.9***	***± 1.4***	***± 50.2***	***± 31.8***
§ 3. mean	**9171 5****	**106.0****	**359.5****	**101****	**282.0****	**493.5****	**1219.5****	**417.0****	**7704 5****	**1027.0****	**1512.0****	**1767.0****
± *SD*	***± 587.6***	***± 11.3***	***± 4.9***	***± 28.3***	***± 1.4***	***± 58.7***	***± 50.2***	***± 106.1***	***± 297.7***	***± 0.0***	***± 19.8***	***± 38.2***

§ = information on respective positive control is reported in Material and Method section A.2. 3 Concentrations were used per plate, 1 = low, 2 = middle, 3 = high.

* = 0.01 ≤ P < 0.05; ** = P < 0.01 (One sided t-test assumes Test > Control)

Table B.6.8.1.1.7.3-2: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 1988), second experiment, plate-incorporation test

Experiment 2: Standard plate test (SPT, plate-incorporation method)												
Strain	**TA 1535**		**TA 1537**		**TA 1538**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control												
Water mean	13.2	14.4	11.4	10.6	11.0	18.2	22.2	23.4	101.8	102.2	197.4	196.8
± *SD*	*± 3.0*	*± 5.6*	*± 2.3*	*± 2.6*	*± 5.1*	*± 1.9*	*± 4.6*	*± 5.7*	*± 13.0*	*± 13.2*	*± 4.1*	*± 15.4*
Test item [µg/plate]												
1.6 mean	17.7	11.0	6.3	6.7	12.0	17.7	15.0	18.7	90.7	109.0	174.0	215.0
± *SD*	*± 8.0*	*± 2.0*	*± 1.5*	*± 3.8*	*± 5.3*	*± 12.5*	*± 2.6*	*± 5.0*	*± 2.5*	*± 7.8*	*± 20.0*	*± 19.3*
8.0 mean	**19.0***	20.3	12.7	8.0	11.7	14.0	20.3	23.7	104.3	112.7	170.0	**218.0***
± *SD*	***± 2.0***	*± 12.3*	*± 3.5*	*± 7.0*	*± 2.1*	*± 4.4*	*± 7.0*	*± 8.4*	*± 14.4*	*± 18.8*	*± 13.1*	***± 7.5***
40 mean	**18.0***	11.7	11.7	14.3	15.3	20.7	28.0	18.7	96.3	116.3	201.7	**229.7***
± *SD*	***± 3.5***	*± 4.6*	*± 2.1*	*± 6.7*	*± 7.8*	*± 5.7*	*± 6.1*	*± 6.8*	*± 6.5*	*± 8.3*	*± 5.0*	***± 7.4***
200 mean	16.7	6.3	12.3	11.7	9.7	13.3	19.3	17.7	102.3	109.3	185.3	211.0
± *SD*	*± 3.2*	*± 2.5*	*± 3.2*	*± 2.1*	*± 1.5*	*± 3.1*	*± 5.8*	*± 1.5*	*± 22.0*	*± 13.7*	*± 6.0*	*± 19.5*
1000 mean	17.7	11.3	12.7	7.0	12.0	11.7	22.7	14.7	107.7	121.7*	187.3	**230.0****
± *SD*	*± 4.5*	*± 4.2*	*± 6.4*	*± 4.6*	*± 4.6*	*± 1.5*	*± 5.0*	*± 2.9*	*± 17.9*	*± 11.8*	*± 7.2*	***± 5.6***
5000 mean	**19.3****	10.3	10.3	5.3	9.3	12.3	19.7	19.0	110.7	95.0	184.7	197.3
± *SD*	***± 0.6***	*± 1.5*	*± 5.9*	*± 0.6*	*± 6.4*	*± 1.5*	*± 5.6*	*± 3.0*	*± 10.4*	*± 7.2*	*± 11.0*	*± 19.6*
Positive control												
§ 1. mean	**20.0***	**49.5****	**58.5****	**23.5****	**90.0****	**49.5****	**129.0****	**52.0****	**200.5****	**167.5****	**290.0***	**231.5***
± *SD*	***± 5.7***	***± 4.9***	***± 7.8***	***± 3.5***	***± 18.4***	***± 2.1***	***± 2.8***	***± 0.0***	***± 10.6***	***± 16.3***	***± 82.0***	***± 31.8***
§ 2. mean	**450.5****	**84.0****	**114.0***	**37.0****	**161.0***	**195.0***	**495.5****	**217.0***	**2913.5***	**455.0****	**996.5****	**953.5****
± *SD*	***± 275.1***	***± 7.1***	***± 4.2***	***± 1.4***	***± 33.9***	***± 43.8***	***± 40.3***	***± 2.8***	***± 494.3***	***± 35.4***	***± 194.5***	***± 98.3***
§ 3. mean	**8244.5***	**142.5***	**217.0***	**101.0***	**506.0***	**495.5***	**1043.0***	**650.5***	**7668.0***	**1180.0***	**1843.5***	**1709.5***
± *SD*	***± 507.0***	***± 4.9***	***± 21.2***	***± 0.0***	***± 35.4***	***± 171.8***	***± 58.0***	***± 37.5***	***± 302.6***	***± 96.2***	***± 65.8***	***± 88.4***

§ = information on respective positive control is reported in Material and Method section I.A.2. 3 Concentrations were used per plate, 1 = low, 2 = middle, 3 = high.

* = 0.01 ≤ P < 0.05; ** = P < 0.01 (One sided t-test assumes Test > Control)

Table B.6.8.1.1.7.3-3: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 1988), third experiment, plate-incorporation test

Experiment 3: Standard plate test (SPT, plate-incorporation method)		
Strain	**TA 1537**	
Metabolic activation	**- S9**	**+ S9**
Vehicle control		
Water mean	5.6	8.2
± *SD*	*± 1.8*	*± 2.2*
Test item		
1.6 mean	11.7	8.7
± *SD*	*± 9.1*	*± 3.2*

Table B.6.8.1.1.7.3-3: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 1988), third experiment, plate-incorporation test

Experiment 3: Standard plate test (SPT, plate-incorporation method)		
Strain	**TA 1537**	
Metabolic activation	**- S9**	**+ S9**
8.0 mean	6.7	7.0
± *SD*	± *3.8*	± *3.5*
40 mean	9 3	9.0
± *SD*	± *6.7*	± *3.6*
200 mean	4.0	6.3
± *SD*	± *1.0*	± *1.5*
1000 mean	5.7	9.3
± *SD*	± *0.6*	± *0.6*
5000 mean	7.0	7.3
± *SD*	± *5.3*	± *2.1*
Positive control		
§ 1. mean	**17.5****	**68.0****
± *SD*	***± 0.7***	***± 8.5***
§ 2. mean	**33.5****	**137.0****
± *SD*	***± 4.9***	***± 9.9***
§ 3. mean	**75.0****	**403.0****
± *SD*	***± 2.8***	***± 35.4***

§ = information on respective positive control is reported in Material and Method section I.A.2. 3 Concentrations were used per plate, 1 = low, 2 = middle, 3 = high.
** = P < 0.01 (One sided t-test assumes Test > Control)

III. CONCLUSIONS

In conclusion, under the experimental conditions reported, the test item is not mutagenic in the Ames test (standard plate test / plate-incorporation method) with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Although statistical significance in the mean number of revertant colonies was observed for all strains at single test item concentrations in the presence or absence of metabolic activation, none of the observations was consistent in independent experiments or showed a dose-response relationship. For strain TA 1537 in the first experiment, the increase in the mean number of revertant colonies was up to 2-fold over control conditions (200 µg/plate) in the absence of S9 mix and up to 1.9-fold over control conditions (8 and 40 µg/plate) in the presence of S9 mix. In both cases, the responses were only of limited dose-response relationship and of limited statistical significance ($p > 0.01$). Besides, the effects were not reproducible in two further experiments with this strain. Considering all available data of the present study, there was no consistent, statistically significant or biologically relevant increase in the mean number of his^+ or trp^+ revertant colonies observed for any tester strain up to the highest concentration of 5000 µg/plate. Thus, the test item was considered negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535, TA 1537 and TA1538 and *E. coli* WP2 uvrA pKM101) with and without metabolic activation.

The study was performed in compliance with GLP and according to OECD guideline 471 (1983). There were only minor deviations when compared to the currently valid OECD guideline 471 (1997), which were considered to not compromise the outcome and the validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

It is not agreed with the applicant that, in the present study, aminomethyl phosphonic acid was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535, TA 1537 and TA 1538 and *E. coli* WP2 uvrA) in the Ames standard plate test with and without metabolic activation. Especially with regard to *E. coli*

WP2 uvrA it is noted that significant differences in the number of revertants were observed in both experiments under conditions of metabolic activation. A clear dose-response relationship is indeed not seen, however, the no conclusions on the biological relevance of the observations can be made also taking into account that no historical control data are available. Therefore, the results of this study are considered equivocal.
The study was considered acceptable in the previous evaluation (RAR, 2015).

Study 4

1. Information on the study

Data point:	CA 5.8.1/022
Report author	[redacted]
Report year	1980
Report title	CP50435: Microbial Mutagenicity Study
Report No	ET-80-402
Document No	NA
Guidelines followed in study	No guideline followed, the study was conducted similarly to OECD 471 (1983)
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Aminomethylphosphonic acid (CP50435, batch: not reported, purity: 99 %), metabolite of glyphosate was investigated for gene mutation in bacteria in an Ames test. *S. typhimurium* strains TA 98, TA 100, TA 1535, TA1537 and TA 1538, and *E. coli* strain WP2 hcr were exposed to the test item, solvent (water) and appropriate positive controls in the presence and absence of metabolic activation (Aroclor-induced rat liver S9 fraction). A single experiment (plate-incorporation method) was performed, with duplicate cultures, using test item concentrations in the range of 10 – 5000 µg/plate. After incubation at 37 °C for two days, the number of bacterial colonies (his^+ or trp^+ revertants) were counted.
Short description of results:	Evaluation of precipitation and cytotoxicity were not included in the study report. However, when comparing the number of revertant colonies of test item treated plates to vehicle controls, there was no evidence for cytotoxicity. The test item did not induce a statistically significant increase in the number of his^+ or trp^+ revertants in any of the tester strains at any concentration when compared to solvent controls, neither in the presence nor in the absence of metabolic activation. The positive controls markedly increased the number of revertant colonies in all strains, demonstrating the functionality of the S9 fraction and the sensitivity of the test system. Based on the experimental results and under the conditions of the test, aminomethylphosphonic acid, metabolite of glyphosate, is negative for gene mutation in bacteria (Ames test) with and without metabolic activation.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable due to a number of guideline deviations when compared to OECD guideline 471 (1997). Only a single experiment was performed without giving a justification for the missing confirmatory experiment. Instead of *E. coli* strain WP2 uvrA, strain WP2 hcr was used. In addition, 2-aminoanthracene was used as sole positive control substance in the presence of S9 mix. Historical control data were not provided and evaluation of cytotoxicity and precipitation were not reported. Acceptance and evaluation criteria were not specified in the study report. The study is therefore considered not valid.
Reasons why the study report is not available for submission	**Conclusion GRG:** Not valid (Category 3b) **Conclusion AGG:** The study is considered to be not acceptable. The study was not accepted in the previous evaluation either (RAR, 2015).

Note AGG:
Since the study was considered to be unreliable no full summary was provided by the applicant. For completeness sake the result table from the study report has been included below to support the claim that no increase in the number of revertants were observed.

Table 2 Reverse mutation tests with or without a liver metabolic activation system (S-9 mix)

Compound	µg/plate	S-9 Mix	No. of revertant colonies / plate					
			WP2 hcr	TA1535	TA100	TA1537	TA1538	TA98
Control (H_2O)		-	13	6	96	5	11	29
			10	8	98	6	6	31
CP50435	10	-	18	4	120	8	7	16
			18	6	124	7	3	30
	50	-	11	4	129	1	7	27
			14	2	141	1	9	30
	100	-	20	8	108	6	4	21
			9	3	137	7	4	17
	500	-	22	11	106	5	7	16
			14	4	124	4	9	30
	1000	-	20	6	84	4	7	24
			14	2	138	8	6	26
	5000	-	12	15	107	4	8	23
			13	6	81	8	5	31
Control (H_2O)		+	12	4	102	10	10	16
			6	5	107	4	13	22
CP50435	10	+	11	7	102	2	6	13
			11	9	105	9	14	19
	50	+	12	5	91	2	10	20
			8	2	91	5	4	12
	100	+	16	7	81	14	7	19
			10	5	83	1	7	19
	500	+	10	5	79	3	5	21
			21	4	103	7	13	16
	1000	+	11	3	97	9	9	14
			21	5	96	5	9	16
	5000	+	17	2	83	4	6	12
			11	4	99	4	7	17
Positive control	10	-	9	10	117	22	8	38
			12	4	111	18	15	41
Positive control (2-amino-anthracene)	10	+	52	164	>3000	128	>3000	>3000
			50	232	>3000	204	>3000	>3000
Positive control		-	1304[a]	694[b]	588[c]	>10000[d]	>3000[e]	212[f]
			1476	684	648	>10000	>3000	223

a) AF-2 0.25 µg/plate b) ß-propiolactone 50 µg/plate
c) AF-2 0.05 µg/plate d) 9-aminoacridine 200 µg/plate
e) 2-nitrofluorene 50 µg/plate f) AF-2 0.1 µg/plate

Study 5

1. **Information on the study**

Data point	CA 5.8.1/023
Report author	[redacted]
Report year	1993
Report title	Mutagenicity test: In vitro Mammalian Cell Gene Mutation Test performed with Mouse Lymphoma Cells (L5178Y). Test compound: AMPA, batch 286-JRJ-73-4.
Report No	13270
Document No	NA
Guidelines followed in study	OECD 476 (1983), US CFR part 700 (F) § 798.5300 (1987)
Deviations from current test guideline (OECD 490, 2016)	The newly introduced cytotoxicity parameters RTG (relative total growth), SG (suspension growth) and RSG (relative suspension growth) in OECD TG 490 (2016) could not be re-calculated, since no data on suspension growth were generated. In the current study, cytotoxicity was evaluated based on cloning efficiency after treatment (CE_1, survival) and after selection (CE_2, viability), in accordance with the previous guideline version. Although the growth rates of the cultures were monitored, the data were not provided within the study report. Further, the number

	of cells treated was below 6 x 10^6 cells, the number of cells recommended in OECD 490 (2016). In several instances, vehicle control cultures exceeded spontaneous levels recommended by OECD guideline 490 (2016). pH and osmolality changes were not assessed. Acceptance criteria specified in the current guideline were not applied and evaluation criteria were based solely on statistics but not on GEF. Although GEF was applied retrospectively, no linear trend was assessed. It was indicated in the report that statistical analysis were conducted, however, they were not clearly reported. In addition, no historical control data were provided for the negative and the positive controls.
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).

2. Full summary

Executive summary

AMPA, metabolite of glyphosate, was tested in a Mouse Lymphoma assay for its ability to induce forward mutations in mammalian cells *in vitro*. In two independent experiments, duplicate cultures of Mouse Lymphoma L5178Y $TK^{+/-}$ cells were exposed to the test item, medium or appropriate positive controls (75 and 100 µg/mL ethylnitrosourea (ENU) without S9 mix and 5 and 10 µg/mL dimethylbenzanthracene (DMBA) with S9 mix). The assay was conducted in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction).

After 3 hours of exposure with S9 mix or 4 hours of exposure without S9 mix, the cells were incubated for 2 - 3 days to allow expression of the mutant phenotype. The expression period was followed by a selection period, in which the cells were cultivated in selection medium containing trifluorothymidine (TFT) for a period of 10 days. Cell survival and cell viability were assessed as cloning efficiency 1 and cloning efficiency 2 at the end of the exposure period and after the expression period, respectively.

Precipitation of the test item in the medium was not reported and there was no cytotoxicity observed up to the highest tested concentration, neither in the presence nor in the absence of S9 mix. Upon treatment with AMPA there was no statistically significant increase in the number of mutant colonies observed in any of the experiments at any concentration, neither in the presence nor in the absence of S9 mix. Mutant frequencies of the medium control cultures were in the expected range. The positive controls ethylnitrosourea and dimethylbenzanthracene markedly increased the mutation frequency, thus demonstrating functionality of the metabolic activation system and the sensitivity of the test.

Based on the results of the present study, AMPA, metabolite of glyphosate, did not induce mutant frequencies in L5178Y $TK^{+/-}$ cells in the presence or absence of S9-mix and is therefore considered non-mutagenic for mammalian cells *in vitro*.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	AMPA
Description:	White powder
Lot/Batch number:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark) was guaranteed for at least 3 years. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	Culture medium

2. Control material:

Negative control:	Untreated cell cultures which were cultivated in cultivation medium only were included in each experiment.
Solvent (vehicle) control:	As culture medium was used as solvent for the test item, the solvent control represents actually the negative control.
Positive control:	- S9 mix: Ethylnitrosourea (ENU), 75 and 150 µg/mL + S9 mix N-nitrosodimethylamine (DMBA), 5 and 10 µg/mL

3. Metabolic activation:

S9 mix was obtained from the livers of Wistar rats weighing approximately 200 g. The rats received a single intraperitoneal injection of Aroclor-1254 at a dose of 500 mg/kg bw. The animals were sacrificed for preparation of liver homogenates 5 days after treatment following a 16 hour period of fasting. The S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factors.

S9 mix component	Concentration	Unit
Hepes buffer, 1M (pH 7.2)	20	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	3	mM
NADP	4	mM
$MgCl_2$	5	mM
S9	12	% (v/v)

4. Test organism:

L5178Y $TK^{+/-}$ mouse lymphoma cells were used. Stocks were maintained in liquid nitrogen and thawed immediately before use. Each batch used for mutagenicity testing was checked for general morphology, growth characteristics and absence of mycoplasma.

5. Cell culture media:

Cultivation medium:	RPMI 1640 medium, supplemented with 10 % horse serum, 200 µg/mL sodium pyruvate and 50 µg/mL gentamicin
Pre-treatment medium A ("THMG medium"):	Cultivation medium supplemented with 9 µg/mL hypoxanthine, 15 µg/mL methotrexate and 22.5 µg/mL glycine
Pre-treatment medium B / treatment medium ("THG medium"):	Cultivation medium supplemented with 50 % conditioned medium, 9 µg/mL hypoxanthine and 22.5 µg/mL glycine
Selection medium:	Cultivation medium, supplemented with 10 % horse serum and 4 µg/mL trifluorothymidine (TFT)
Incubation:	At 37 °C and 5 % CO_2

6. Locus examined: Thymidine kinase (TK)

7. Test concentrations and number of replicates:
The dose-range was selected on the basis of a preliminary toxicity test (data not provided in study report) in which the cell-growth was measured for a period of 2-3 days after exposure. If possible the maximum dose was chosen so that the survival (cloning efficiency) was approximately 20 % and so that the cells grew at a normal rate before the end of the 3 day period.

First experiment			
Metabolic activation	**Duration of exposure**	**Concentrations**	**Replicates**
-S9 mix	4 h	0.31, 0.63, 1.3, 2.5 and 5.0 mg/mL	Duplicate
+S9 mix	3 h	0.31, 0.63, 1.3, 2.5 and 5.0 mg/mL	Duplicate
Second experiment			
Metabolic activation	**Duration of exposure**	**Concentrations**	**Replicates**
-S9 mix	4 h	0.63, 1.3, 2.5 and 5.0 mg/mL	Duplicate
+S9 mix	3 h	0.63, 1.3, 2.5 and 5.0 mg/mL	Duplicate

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 14 Dec 1992 – 21 Jan 1993
Finalisation date: 18 Feb 1993

2. Mutation assay:
Pre-treatment of cells:
Thawed cells were maintained at a density of 2 x 10^5 - 1.5 x 10^6 in sterile NUNC plastic flasks and incubated at 37 °C and 5 % CO_2. Prior to treatment, spontaneous TK deficient mutants ($TK^{-/+}$ cells) were eliminated from the stock cultures by incubating the cells for 1 - 2 days in THMG medium (pre-treatment medium A), followed by a recovery period of 1 - 3 days in THG medium (pre-treatment medium B).

Treatment:
Two independent experiments using duplicate cultures per condition were performed in the presence and absence of metabolic activation.
For treatment of cells in the absence of S9 mix, cell suspensions of 15 mL containing 3 x 10^5 cells/mL (i.e. 4.5 x 10^6 cells in total) were mixed with 1 mL test item solution, resulting in final concentrations in the range of 0.31 – 5.0 mg/mL (first experiment) and 0.63 – 5.0 mg/mL (second experiment). Afterwards the cultures were incubated for 4 hours at 37 °C under gentle shaking.
For treatment of cells in the presence of S9 mix, cell suspensions of 2 mL containing 2.25 x 10^6 cells were mixed with 0.5 mL S9 mix and 0.5 mL of test item solutions, resulting in final concentrations in the range of 0.31 – 5.0 mg/mL (first experiment) and 0.63 – 5.0 mg/mL (second experiment). The cultures were incubated for 3 hours at 37 °C under gentle shaking.
At the end of the exposure period, the cells were centrifuged, re-suspended in 15 mL fresh medium and a small sample of cells from each culture was diluted and seeded in a microtiter plate at a density of 2 cells/well for determination of relative cell survival (cloning efficiency 1).

Expression period:
After the exposure period, the cells were incubated for a 2 - 3 days expression period, in which each culture was counted daily, diluted to 3 x 10^5 cells/mL and the growth rate was recorded. After the expression period, each culture was divided. One aliquot of each culture was plated to determine the cell viability (cloning efficiency 2) of the cultures, the other one was used for the selection of mutants.

Selection period:
For the selection of mutants, two microtiter plates were prepared from each post-expression culture, seeding 2000 cells per well in medium supplemented with 4 µg/mL trifluorothymidine (TFT). After an incubation period of 10 days, the number of cell clones was counted. The clones were differentiated into large clones and small dense clones. Small colonies were considered to be associated with clastogenic effects, large colonies were considered to be associated with gene mutation effects.

3. Cytotoxicity:
Cloning efficiency (CE_1 survival)

At the end of the exposure period, a sample of each cell culture was collected to assess cell survival. A full 96-well microtiter plate was seeded at a density of 2 cells/well for each culture. After 10 days of incubation, the number of colonies was counted.

CE_2 (viability)
After the expression period, 2 - 3 days after end of exposure, a sample of each cell culture was collected to assess cell viability. For each culture, a full 96-well microtiter plate was seeded at a density of 2 cells/well. After 10 days of incubation, the number of colonies was counted.

4. Evaluation:
Cytotoxicity (cloning efficiency CE)
The number of colonies divided by the number of cells plated was calculated for each sample. The absolute cloning efficiency was determined for each test group, as well as the relative cloning efficiency in comparison to the solvent control group.

CE_1 (survival)
The cytotoxicity of the test substance after the exposure period was determined for each test group and is indicated as absolute and relative cloning efficiency (CE_1 and RCE_1, respectively).

CE_2 (viability)
The cytotoxicity of the test substance at the end of the expression period was determined for each test group and is given as absolute and relative cloning efficiency (CE_2 and RCE_2, respectively).
The cloning efficiency (CE, %) was calculated for each test group as follows:

$$CE_x = -\frac{1}{2}\ln\frac{\text{Number of empty wells}}{\text{Number of wells seeded}}$$

$$RCE_x = \frac{CE_x \text{ of the test group}}{CE_x \text{ of the negative or vehicle control}} \times 100$$

Mutant frequency (MF)
The number of empty wells and the number of wells containing colonies were scored and reported. The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage).

Uncorrected mutant frequency:
The uncorrected mutant frequency per 10^6 cells (MF_{uncorr}) was calculated for each test group as follows:

$$MF_{uncorr.} = -\frac{1}{2000}\ln\frac{\text{Number of empty wells}}{\text{Number of wells seeded}}$$

Corrected mutant frequency
The corrected mutation frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$MF_{corr.} = \frac{MF_{uncorr.}}{CE_2} \times 100$$

Determination of borderline mutant frequency based on GEF
The GEF (global evaluation factor) method requires that the MF exceeds a value based on the global distribution of the background MF of the test method. This value is defined as the mean of the negative/vehicle MF distribution plus one standard deviation.
Based on a large data base (n = 493 experiments) from six laboratories a GEF of 126 mutant colonies per 10^6 cells [mean MF_{corr} = 99 × 10^{-6} colonies; standard deviation = 27 × 10^{-6} colonies] was calculated for the microwell method. To be judged positive, the mutation frequency has to exceed a threshold of 126 colonies per 10^6 cells (GEF) above the concurrent negative/vehicle control value. The borderline mutant frequency was calculated for each experiment separately as follows:
Borderline MF = $MF_{\text{vehicle control corr}}$ + GEF (126 × 10^{-6}). The borderline MF was not evaluated as part of the present study, but was determined retrospectively for this evaluation.

5. Statistics:
Statistical analysis was conducted using the Analysis of Variance method on the corresponding test and control cultures.

6. Acceptance criteria:
Acceptance criteria were not defined in the study report.

7. Evaluation criteria:
A substance was considered to be mutagenic if the following criteria were met:

- There was a statistically significant and reproducible increase in the mutation frequency as compared to the negative control cultures.
- A dose-response was evident.
- The mutation frequency at the dose level where the highest effect was found was more than twice the concurrent spontaneous mutant frequency.

Sporadic occurring statistically significant increases in revertants which were not dose related (i.e. occurring at the lower dose level when there was no increase at higher non-toxic doses) were considered incidental and not relevant for the evaluation.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the present study.

2. CYTOTOXICITY
There was no significant toxicity observed upon treatment with AMPA up to the highest tested concentration of 5.0 mg/mL.

Table B.6.8.1.1.7.5-1: Results of the MLA - gene mutation assay in mammalian cells with AMPA, metabolite of glyphosate ([redacted], 1993), first experiment

Test group	Mutagenicity data[$]			Toxicity data[$]			
	Corrected Mutant Frequency per 10^6 cells			Cloning efficiency (CE_1 -survival)		Cloning efficiency (CE_2 -viability)	
	total	small	large	absolute	relative (RCE_1)	absolute	relative (RCE_2)
Without metabolic activation; 4-hour exposure period							
Medium control	109	45	57	59.0	100.0	1.3	100.0
MF threshold[§]	235	171	183				
Test item [mg/mL]							
0.31	82	43	35	82.0	139.0	131.5	100.4
0.63	138	65	61	65.0	110.2	135.0	103.1
1.30	132	63	58	82.5	139.8	119.5	91.2
2.50	162	72	77	74.5	126.3	103.0	78.6
5.00	172	89	69	66.0	111.9	98.5	75.2
ENU 75 µg/mL	747	306	251	48.0	81.4	71.0	54.2
IMF	*638*	*262*	*194*				
ENU 150 µg/mL	1177	402	381	36.0	61.0	62.0	47.3
IMF	*1095*	*360*	*346*				
With metabolic activation; 3-hour exposure period							
Medium control	178	87	79	59.5	100.0	82.0	100.0
MF threshold[§]	304	213	205				

Table B.6.8.1.1.7.5-1: Results of the MLA - gene mutation assay in mammalian cells with AMPA, metabolite of glyphosate ([redacted], 1993), first experiment							
Test group	**Mutagenicity data[$]**			**Toxicity data[$]**			
	Corrected Mutant Frequency per 10^6 cells			**Cloning efficiency (CE_1 -survival)**		**Cloning efficiency (CE_2 -viability)**	
	total	**small**	**large**	**absolute**	**relative (RCE_1)**	**absolute**	**relative (RCE_2)**
Test item [mg/mL]							
0.31	118	46	67	77.0	129.4	85.5	104.3
0.63	165	76	77	52.5	88.2	103.5	126.2
1.30	202	82	105	85.5	143.7	78.0	95.1
2.50	170	72	87	65.5	110.1	77.0	93.9
5.00	255	100	139	56.5	95.0	55.0	67.1
DMBA 5 µg/mL	2311	756	859	36.0	60.5	28.0	34.1
IMF	*2133*	*669*	*780*				
DMBA 10 µg/mL	1537	537	518	28.0	47.1	44.0	53.7
IMF	*1420*	*491*	*451*				

IMF: Induced Mutant Frequency, an increase above vehicle MF, IMF should be ≥ 300 x 10^{-6} for total MF or ≥ 150 x 10^{-6} for small colonies
MF: Mutant frequency; [§] = $MF_{vehicle\ control\ corr}$ + GEF (126 x 10^{-6}), rounded
[$] = Mutant frequency values and toxicity data for 10^6 cells. Values differ from those mentioned in study report, where the MF and CE values were given for 10^4 cells.
ENU: Ethylnitrosourea, DMBA: Dimethylbenzanthracene

Table B.6.8.1.1.7.5-2: Results of the MLA - gene mutation assay in mammalian cells with AMPA, metabolite of glyphosate ([redacted], 1993), second experiment							
Test group	**Mutagenicity data[$]**			**Toxicity data[$]**			
	Corrected Mutant Frequency per 10^6 cells			**Cloning efficiency (CE_1 -survival)**		**Cloning efficiency (CE_2 -viability)**	
	total	**small**	**large**	**absolute**	**relative (RCE_1)**	**absolute**	**relative (RCE_2)**
Without metabolic activation; 4-hour exposure period							
Medium control	189	88	87	45.0	100.0	81.0	100.0
MF threshold[§]	315	214	213				
Test item [mg/mL]							
0.61	200	96	87	61.0	135.6	89.0	109.9
1.30	198	91	90	58.5	130.0	86.0	106.2
2.50	225	112	94	47.0	104.4	85.5	105.6
5.00	173	86	75	45.0	100.0	82.0	101.2
ENU 75 µg/mL	1484	396	338	45.0	100.0	84.0	103.7
IMF	*1484*	*396*	*338*				
ENU 150 µg/mL	1337	338	384	28.0	47.5	81.0	61.8
IMF	*1337*	*338*	*384*				
With metabolic activation; 3-hour exposure period							

Table B.6.8.1.1.7.5-2: Results of the MLA - gene mutation assay in mammalian cells with AMPA, metabolite of glyphosate ([redacted], 1993), second experiment							
Test group	**Mutagenicity data[$]**			**Toxicity data[$]**			
	Corrected Mutant Frequency per 10^6 cells			**Cloning efficiency (CE_1 -survival)**		**Cloning efficiency (CE_2 -viability)**	
	total	**small**	**large**	**absolute**	**relative (RCE_1)**	**absolute**	**relative (RCE_2)**
Medium control	164	78	75	56.0	100.0	84.0	100.0
MF threshold[§]	290	204	201				
Test item [mg/mL]							
0.63	87	82	184	60.0	107.1	87.0	103.6
1.30	105	111	236	52.5	93.8	78.5	93.5
2.50	104	100	223	31.0	55.4	79.5	94.6
5.00	91	94	201	38.0	67.9	79.0	94.0
DMBA 5 µg/mL	3356	786	811	22.0	39.3	39.0	46.4
IMF	*3356*	*786*	*811*				
DMBA 10 µg/mL	3479	832	886	18.0	30.3	36.0	43.9
IMF	*3479*	*832*	*886*				

IMF: Induced Mutant Frequency, an increase above vehicle MF, IMF should be ≥ 300 x 10^{-6} for total MF or ≥ 150 x 10^{-6} for small colonies
MF: Mutant frequency; § = $MF_{vehicle\ control}$ corr + GEF (126 x 10^{-6}), rounded
[$] = Mutant frequency values and toxicity data for 10^6 cells. Values differ from those mentioned in study report, where the MF and CE values were given for 10^4 cells.
ENU: Ethylnitrosourea, DMBA: Dimethylbenzanthracene

3. SOLUBILITY

Precipitation of the test item was not reported.

4. MUTANT FREQUENCY

There was no statistically significant increase in mutation frequency observed upon treatment with AMPA in both experiments at any of the tested concentrations, neither in the presence, nor in the absence of metabolic activation. Mutant frequencies of the medium control cultures were in the expected range. The positive controls ethylnitrosourea and dimethylbenzanthracene markedly increased the mutation frequency, thus demonstrating functionality of the metabolic activation system and the sensitivity of the test.

III. CONCLUSIONS

Based on the experimental findings, AMPA, metabolite of glyphosate, did not induce mutant frequencies in L5178Y $TK^{+/-}$ cells in the presence or absence of S9-mix. Under the conditions of the test, the test item is negative for mutagenicity in mammalian cells *in vitro.*

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, AMPA was negative for mutagenicity in L5178Y $TK^{+/-}$ cells with and without metabolic activation.

The study was conducted according to OECD guideline 476 (1983) and in compliance with GLP. When compared to the currently valid OECD test guideline 490 (2016), there were a number of deviations of minor degree. Cytotoxicity was evaluated based on cloning efficiencies, in accordance with the previous guideline version. Mutant frequency and toxicity data given in the study report included data from 10^4 cells. For this evaluation, data were calculated retrospectively for 10^6 cells. In addition, the borderline mutant frequency based on GEF was determined retrospectively. The deviations were considered to not compromise the validity of the study. Therefore, the study was considered valid.

Assessment and conclusion by RMS:
It is agreed with the applicant that, in this study, AMPA was negative for mutagenicity in L5178Y $TK^{+/-}$ cells with and without metabolic activation. However, some deviations from the current guideline (OECD 490, 2016) were noted. It is agreed that certain data were determined retrospectively on the data reported in the study report, however, it is noted that the number of treated cells is below the number of cells to be treated according to OECD 490 (2016). Although the applicant considers this deviation as minor, the RMS is of the opinion that this may have an impact on the study outcome. Overall, the study is considered acceptable but with restrictions (reliable with restrictions).
The study was accepted in the previous evaluation (RAR, 2015).

Study 6

1. Information on the study

Data point	CA 5.8.1/024
Report author	[redacted]
Report year	2002
Report title	Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes using an *in vitro* procedure with AMPA (Aminomethylphosphonic acid)
Report No	IPL-R 020625
Document No	NA
Guidelines followed in study	OECD 482 (1986)
Deviations from current test guideline	Not applicable. OECD 482 was deleted in 2014. When compared to the previous OECD 482 (1986), no deviations were observed.
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Supportive (Category 2a) **Conclusion AGG:** The study is not considered to be acceptable only since the UDS assay is no longer a standard method.

2. Full summary

Executive summary
AMPA, metabolite of glyphosate , was investigated for induction of unscheduled DNA synthesis (UDS) in primary rat hepatocytes *in vitro*. Two independent experiments were performed. Hepatocytes were isolated from the livers of young male Fischer rats and exposed to test item concentrations in the range of 0.625 – 10 mM in medium supplemented with ^{3}H-thymidine. Solvent (medium) and positive controls (2-acetamidofluorene, 6.25 µM) were included in each experiment. After 17 – 20 hours of incubation at 37 °C, slides were prepared for autoradiography. In parallel, cell viability was assessed by MTT assay in separate cultures. A total of 150 cells per condition were scored for nuclear and cytoplasmatic grains and the mean number of net nuclear grains (NNG) per cell was determined to evaluate unscheduled DNA synthesis for each condition. In addition, the percentage of cells in repair and the percentage of cells in S-phase were determined.
Precipitation of AMPA in culture medium was not reported. In addition, there were no changes on osmolality. At 10 mM there was a slight change in pH value with 6.70 vs. 7.26 when compared with the solvent control. Cytotoxicity was observed in the first experiment at 10 mM and in the second experiment at 5 and 10 mM, respectively.
In both experiments, there was no statistically significant or dose-related increase in the mean number of net nuclear grain counts up to the highest tested concentration and no statistically significant increase in the percentage of cells in repair when compared with solvent controls at any tested concentration. Moreover, the frequency of cells in S-phase was low for all conditions.
Values obtained for the solvent and the positive control were within the range of the laboratories historical control data, demonstrating the validity and the sensitivity of the test. Based on the experimental findings and under the conditions of the test, AMPA, metabolite of glyphosate did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminomethylphosphonic acid (AMPA)
Identification: 020404
Description: White crystalline powder
Lot/Batch number: A015478701
Purity: 99.9 %
Stability of test compound: The stability of the test item at storage conditions or in solvent were not specified.

1. **Control material:**
Negative control: The negative control is actually the solvent control.
Solvent (vehicle) control: Culture Medium (William's E medium)
Positive controls: 2-Acetamidofluorene, 6.25 µM in DMSO

3. Hepatocyte isolation:
Primary rat hepatocytes were isolated by *in situ* collagenase perfusion from the livers of young male Fischer rats. As two independent experiments were performed, one rat liver perfusion was used for each experiment. The animals were anesthetised with pentobarbital and a V-shaped incision was made in the abdominal wall of the animals. The liver was perfused via the portal vein at 37 °C with Hepes buffer for 5 minutes, followed by 5 minutes perfusion with collagenase buffer consisting of Hepes buffer with 4 mM $CaCl^{2+}$ and 0.025 % collagenase. Finally, the perfused liver was dissected and the isolated hepatocytes were washed in a centrifugation step. Cell viability was determined using the trypan blue technique.

4. Cell culture:
Cell culture establishment: Freshly isolated hepatocytes were seeded in plating medium at a density of 4.5 x 10^5 cells on coverslips in conventional 6-well plates. The cells were incubated for approx. 90 minutes and allowed to attach.
Plating medium: William's medium E
Treatment medium: William's medium E, supplemented with 10 µCi/mL tritiated thymidine
Incubation: At 37 °C in an atmosphere of 5 % CO_2

5. Test concentrations:

Experiment	Concentrations	Replicates
First experiment:	0.625, 1.25, 2.5, 5 and 10 mM	Triplicates
Second experiment:	0.625, 1.25, 2.5, 5 and 10 mM	Triplicates

B. STUDY DESIGN AND METHODS

(a) Dates of experimental work: 29 Apr – 02 Jul 2002
Finalisation date: 09 Oct 2002

(b) Cytotoxicity:
Cytotoxicity was determined using the MTT assay. 3.5 x 10^4 cells/well were seeded in two separate wells of a conventional 6-well plate. The cells were exposed to the test item, solvent (medium) or positive control (6.25 µM 2-acetamidofluorene) under conditions which were identical to those used for determination of unscheduled DNA synthesis. Test item concentrations of 0.078 – 10 mM were used. After 17 – 20 hours of incubation at 37 °C, the medium was removed and replaced by 2 mL medium containing 0.5 mg/mL MTT, followed by incubation for 2 - 3 hours. Thereafter, the cells were washed with phosphate buffered saline (PBS). The insoluble formozan was solubilised using HCl : isopropoanol (1 : 23) and the absorbance was read at 550 nm for each concentration. Cell viability is expressed as a percentage of the control groups.

(c) Unscheduled DNA synthesis:
Cell treatment:
After cell culture establishment, the medium was aspirated from the cells, the hepatocytes were washed with culture medium and finally exposed to the test item, solvent (medium) or positive control (6.25 µM 2-acetamidofluorene) in ^{3}H-thymidine-enriched medium. Test item concentrations in the range of 0.625 – 10 mM were used. After 17 – 20 hours of incubation at 37 °C, the cells were prepared for autoradiography.

Cell harvest and slide preparation:
After treatment, the slides were coated in Kodak NTB-2 liquid emulsion. After gelling, the slides were

incubated in a light-protected box and kept in the refrigerator for 14 days. Finally, the cells were stained with Harris hemalum, dehydrated in ethanol, cleared in xylene and mounted with coverslips for microscopic examination.

Evaluation:
To assess unscheduled DNA synthesis in hepatocytes, grain counting was performed using an image analysis system. At least 150 cells per condition (3 slides with 50 cells/slide) were scored for nuclear and cytoplasmic grain counts. Only cells with normal morphology were scored. Isolated nuclei with no surrounding cytoplasm, cells with unusual staining artefacts and heavily labelled cells in S-phase were not scored. Net nuclear grains per cell were determined by subtracting the number of cytoplasmatic grain counts from the number of nuclear grain counts.

4. Statistics:
Performance of statistical analysis was not specified in the study report.

5. Acceptance criteria:
The assay was considered valid if the following criteria were met:

- The negative control slides had a group mean net nuclear grain (NNG) value which was in the laboratories historical control range.
- The positive control had a group mean NNG value of not less than 5 NNG counts with 50 % or more cells having NNG counts of 5 or more and the values were statistically significant relative to the solvent control.

6. Evaluation criteria:
The test item was considered positive for unscheduled DNA synthesis (UDS) if the following criteria were met:

- At any concentration tested, group mean net nuclear grain (NNG) values were > 0 and 20 % or more of the cells responded (NNG ≥ 5).
- An increase was seen in both NNG and the percentage of cells in repair.
- A dose related increase was seen in both NNG and the percentage of cells in repair.
- Any induction of UDS was reproduced in an independent experiment.

If a test item failed to induce UDS at any dose in either experiment, it was considered clearly negative in this system.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study.

2. CYTOTOXICITY
Cytotoxicity was observed in the first experiment at 10 mM (84.42 % of control) and in the second experiment at 5 and 10 mM (85.9 and 81.80 % of control), respectively.
Osmolality measurements revealed no changes upon addition of AMPA to the culture medium. There was a slight decrease of the pH at 10 mM with 6.70 vs. 7.26 in the solvent control; however, the value was within the biologically acceptable range of pH (6.5 – 8). Based on these findings, 10 mM was chosen as highest concentration for the unscheduled DNA synthesis assay.

3. SOLUBILITY
Precipitation of the test item in culture medium was not reported.

4. UNSCHEDULED DNA SYNTHESIS
In two experiments, AMPA did not induce a statistically significant or dose-related increase in the mean number of net nuclear grain counts when compared to solvent controls up to the highest tested concentration of 10 mM. In addition, there was no statistically significant increase in the percentage of cells in repair when compared with solvent controls at any tested concentration. Moreover, the frequency of cells in S-phase was low for all conditions.
Values obtained for the solvent and the positive control were within the range of the laboratories historical control data, demonstrating the validity and the sensitivity of the test.

Table B.6.8.1.1.7.6-1: Results of the UDS assay with AMPA, metabolite of glyphosate, first experiment ([redacted], 2002)

	Viability (%)	**NNG**	**NNG of cells in repair**	**% of cells in repair NNG > 5**	**% of cells in S-phase**
Medium control	100.00	-2.38 ± 5.20	5.63 ± 0.51	4.21 ± 1.53	0.0
HCD[#] mean	/	-2.62 ± 2.04	7.22 ± 1.21	7.42	/
range	/	-5.57 - -0.13	5.96 - 9.5	2 - 12	/
Test item [mM]					
0.625	102.66	-3.81 ± 5.29	6.61 ± 1.56	3.89 ± 1.53	0.0
1.250	104.49	-3.61 ± 5.23	6.99 ± 0.56	4.02 ± 1.53	0.0
2.500	104.03	-3.93 ± 5.24	6.83 ± 0.91	5.18 ± 1.53	0.0
5.000	106.78	-3.35 ± 5.10	6.28 ± 1.66	3.45 ± 1.00	0.0
10.000	84.42	-2.04 ± 4.82	6.91 ± 0.74	5.43 ± 1.53	0.3
Positive control 2-AAF					
6.25 µM	128.69	30.81 ± 18.22	31.55 ± 4.84	96.61 ± 2.00	13.9
HCD[#] mean	/	20.29 ± 5.72	20.6 ± 5.60	95.25	/
range	/	11.56 - 30.87	12.87 - 31.72	88 - 100	/

HCD[#]: Historical control data, generated in the testing laboratory in 1999 - 2000 (5 assays)
NNG: Net nuclear grain count

Table B.6.8.1.1.7.6-2: Results of the UDS assay with AMPA, metabolite of glyphosate, second experiment ([redacted], 2002)

	Viability (%)	**NNG**	**NNG of cells in repair**	**% of cells in repair NNG > 5**	**% of cells in S-phase**
Medium control	100.00	-4.612 ± 5.81	5.37 ± 0.21	3.74 ± 2.08	0.3
HCD[#] mean	/	-2.62 ± 2.04	7.22 ± 1.21	7.42	/
range	/	-5.57 - -0.13	5.96 - 9.5	2 - 12	/
Test item [mM]					
0.625	96.89	-4.77 ± 5.46	6.90 ± 0.60	1.78 ± 0.00	0.7
1.250	94.80	-4.04 ± 5.35	6.87 ± 1.00	4.49 ± 1.53	0.5
2.500	91.34	-3.81 ± 5.82	7.07 ± 1.61	6.18 ± 2.08	0.2
5.000	85.90	-3.47 ± 5.83	7.83 ± 0.29	7.73 ± 1.73	0.6
10.000	81.78	-5.58 ± 6.06	6.97 ± 1.29	3.76 ± 1.53	0.0
Positive control 2-AAF					
6.25 µM	88.89	17.57 ± 11.18	17.73 ± 3.29	94.94 ± 9.45	0.0
HCD[#] mean	/	20.29 ± 5.72	20.6 ± 5.60	95.25	/
range	/	11.56 - 30.87	12.87 - 31.72	88 - 100	/

HCD[#]: Historical control data, generated in the testing laboratory in 1999 - 2000 (5 assays)
NNG: Net nuclear grain count

III. CONCLUSIONS

Under the conditions of the test, AMPA, metabolite of glyphosate did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In the present study, AMPA was negative for unscheduled DNA synthesis in primary rat hepatocytes *in vitro*. The study was performed under GLP conditions and in accordance with OECD guideline 482 (1986), which was deleted in 2014. As the Unscheduled DNA Synthesis (UDS) assay is no longer a standard method described by current guidelines, the study was considered to provide supporting information.

Assessment and conclusion by RMS:

It is agreed with the applicant's conclusion that, in this test, AMPA was negative for unscheduled DNA synthesis in primary rat hepatocytes *in vitro*. Considering that the UDS assay is no longer a standard method, the study was considered not acceptable though.
The study was accepted in the previous evaluation (RAR, 2015).

Study 7

1. Information on the study

Data point	CA 5.8.1/025
Report author	[redacted]
Report year	1991
Report title	Evaluation of the potential of AMPA to induce unscheduled DNA synthesis in the *in vitro* hepatocyte DNA repair assay using the male F-344 rat.
Report No	SR-91-234
Document No	Not reported
Guidelines followed in study	Similar to OECD 482 (1986)
Deviations from current test guideline	Not applicable. OECD 482 was deleted in 2014. There were no deviations when compared to the previous OECD 482 (1986). It is noted, however, that no historical control data were included in the study report but this was not a requirement according to OECD 482.
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 not considered to be acceptable only since the UDS assay is no longer a standard method.

2. Full summary

Executive summary

AMPA, metabolite of glyphosate, was tested for induction of DNA repair (unscheduled DNA synthesis, UDS) in primary rat hepatocytes *in vitro*. Two independent experiments were performed. Hepatocytes were isolated from the livers of adult male F344 rats and the cells were exposed to test item concentrations in the range of 5 to 5000 µg/mL in medium supplemented with radiolabeled tritiated thymidine (^{3}H-TdR). In the first experiment, 8 concentration steps were used whereas 10 concentration steps were used in the second experiment. Solvent (medium) and positive controls (3 µg/mL 2-acetylaminofluorene) were included in each experiment. After 19 hours of incubation at 37 °C, the cells were visually inspected for cytotoxicity, processed for slide preparation and 90 cells per condition (30 per slide) were evaluated for UDS. UDS was quantified by determining the net increase in nuclear grain counts and the number of cells in repair.
Precipitation of the test item in solvent was not observed. Cytotoxicity was noted at 5000 µg/mL in the first experiment and at 3800 and 5000 µg/mL in the second experiment.
In both experiments, AMPA did not induce significant increases in the mean number of net nuclear grain counts in rat hepatocytes when compared to the solvent control at any of the tested concentrations. In addition, the percentage of cells in repair was ≤ 6 %. Values obtained for the solvent and the positive control were in the expected range, demonstrating the validity and the sensitivity of the test system.
Under the conditions of the test and based on the results of the present study, AMPA, metabolite of glyphosate, did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: AMPA

Identification:	Not specified
Description:	White solid
Lot/Batch number:	HET-9001-1463-T
Purity:	94.38 %
Stability of test compound:	The stability of the test item at storage conditions was guaranteed until the expiry date Jan 1993.

2. Control material:

Negative control:	Untreated cell cultures which were cultivated in cultivation medium only were included in each experiment.
Solvent (vehicle) control:	As culture medium was used as solvent for the test item, the solvent control represents actually the negative control.
Positive controls:	2-Acetylaminofluorene (2-AAF), 3 µg/mL in DMSO

3. Hepatocyte isolation:

Primary rat hepatocytes were isolated by *in situ* collagenase perfusion from the livers of adult male F344 rats. A total of two rats were used in the study, using one rat for each experiment. The rats were approximately 15 – 19 weeks old and weighed 315.4 and 345.6 g. The animals were anesthetised with 60 mg/kg bw pentobarbital, perfused with a collagenase solution and the liver lobes were combined to isolate hepatocytes from the perfused livers. Finally, the cell viabilities were determined prior to plating.

4. Cell culture:

Cell culture establishment:	Freshly isolated hepatocytes were seeded in plating medium at a density of 3 x 10^5 cells. The cells were inoculated into numbered six-well culture dishes containing coverslips. The cells were allowed to attach for 1.5 - 2 hours and afterwards washed with culture medium.
Plating medium:	William's medium E, supplemented with 10 % fetal bovine serum, 2 mM L-glutamine and 50 µg/mL gentamycin
Treatment medium:	William's medium E, supplemented with 2 mM L-glutamine, 50 µg/mL gentamicin and 10 µCi/mL tritiated thymidine (^{3}H-TdR, 80 Ci/mmol)
Incubation:	At 37 °C in a humidified atmosphere containing 5 % CO_2.

4. Test concentrations:

Experiment	Concentrations	Replicates
First experiment:	5, 10, 50, 100, 500, 1000, 2500 and 5000 µg/mL	Triplicate
Second experiment:	5, 10, 50, 100, 250, 500, 1000, 2500, 3800 and 5000 µg/mL	Triplicate

B. STUDY DESIGN AND METHODS

Dates of experimental work: 20 Jun – 18 Sep 1991
Finalisation date: 04 Dec 1991

1. Cytotoxicity

Cytotoxicity of the test substance was assessed via visual inspection by light microscopy. Cultures were characterised as "good" (normal cell morphology, sufficient attachment of cells to easily permit scoring of cells), "sparse" (most cells showed normal morphology but attachment of cells was < 25 % of control attachment), "pyknotic nuclei" (a large proportion of cells had small, darkly stained nuclei) or "cytotoxic" (few or no cells present on coverslips, or virtually all cells had pyknotic nuclei or other obvious morphologic effects such as damaged cell membranes or absence of cytoplasm).

6. Unscheduled DNA synthesis

Cell treatment:

Two independent experiments using hepatocytes of different rats were performed. The purpose of the first experiment was to determine cytotoxicity and to interpret any UDS response. On the basis of the cytotoxicity results from the first experiment, a second experiment was performed to confirm the results of the first. After cell culture establishment, triplicate cultures of the hepatocytes were cultivated in ^{3}H-TdR enriched treatment medium. In the first experiments, hepatocytes were exposed for 19 hours at 37 °C to eight test item

concentrations in the range of 5 to 5000 µg/mL. In the second experiment, the cells were treated under identical conditions to ten test item concentrations in the range of 5 to 5000 µg/mL. In both experiments, solvent and positive controls (3 µg/mL 2-acetylaminofluorene) were included. After exposure, all cultures were washed with culture medium and processed for slide preparation.

Cell harvest and slide preparation:
Immediately following treatment, the cells were swelled in 1 % sodium citrate solution, fixed in 1:3 glacial acetic acid / ethanol and washed with deionised water. The coverslips were mounted on slides and dipped in Kodak NTB-2 photographic emulsion for 7 days at -20 °C before development. Subsequently, cells were stained with 1 % methyl-green pyronin Y, dried, coverslipped and evaluated.

Evaluation:
Primary hepatocyte DNA repair was determined by quantitative audioradiographic grain counting. The net increase in nuclear grains was quantified for at least 30 morphologically unaltered cells on a randomly selected area of each slide. Three slides per condition were scored. The higher count from two nuclear sized areas over the most heavily labeled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to give the net grains per nucleus. The percentage of cells in repair (cells showing at least 5 nuclear grains) was calculated for each concentration.

4. Statistics:
No statistical methods were used for the evaluation of data.

5. Acceptance criteria:
Data were considered acceptable if the mean number of net grains per nucleus and the percentage of cells in repair values of the solvent control were within the normal range of the laboratories historical control data and if the positive control produced an unscheduled DNA response of greater than 5.0 net grains per nucleus.

3. Evaluation criteria:
A substance was considered unequivocally positive for unscheduled DNA synthesis if the mean net grain count for any dose was greater than 5.0 nuclear grains per cell.
A substance was considered unequivocally negative if the mean net grain count was less than 0 nuclear grains per cell and the percentage of cells in repair was less than 10 % for all groups.
When results fell within 0 to 5 nuclear grains per nucleus or when the percentage of cells in repair exceeded 10 %, the presence of a dose response, the frequency distribution of cellular responses, increases in percentage of cells in repair and reproducibility data among concentrations were considered.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS
Analytical determinations have not been performed in the study. Solubility tests with AMPA in culture medium, DMSO, acetone and ethanol were performed prior to the study. Culture medium allowed the highest possible test concentration of 5000 µg/mL and was selected as solvent for the unscheduled DNA synthesis assay.

2. CYTOTOXICITY
Cytotoxicity was observed at 5000 µg/mL in the first experiment and at 3800 and 5000 µg/mL in the second experiment. The corresponding slides could not be scored for net nuclear grains.

3. SOLUBILITY
Precipitation of the test item in culture medium was not reported.

4. UNSCHEDULED DNA SYNTHESIS
In both experiments, AMPA did not induce significant increases in the mean number of net nuclear grain counts in rat hepatocytes when compared to those of the solvent control at any tested concentration. In triplicate cultures per condition, the values for nuclear grains per nucleus were negative and the percentage of cells in repair was ≤ 6 % up to the highest concentration tested.
Values obtained for the solvent and the positive control were as expected, demonstrating the validity and the sensitivity of the test system.

Table B.6.8.1.1.7.7-1: Results of the UDS assay with AMPA, first experiment ([redacted], 1991)

First experiment

	Net grains / nucleus Mean ± SE	Median	% of cells in repair
Medium control	-15.3 ± 0.7	-14.9	3
Test item [µg/mL]			
5.00	-17.3 ± 1.4	-17.6	0
10.00	-14.1 ± 2.5	-16.2	6
50.00	-13.9 ± 3.1	-12.2	1
100.00	-17.8 ± 3.1	-17.6	2
500.00	-18.6 ± 2.7	-18.9	0
1000.00	-13.1 ± 2.2	-13.5	0
2500.00	-12.9 ± 1.8	-12.2	1
5000.00	Toxic[#]	-	-
Positive control 2-AAF [3 µg/mL]	9.4 ± 3.7	10.8	63

2-AAF: 2-acetylaminofluorene
SE: Standard errors, represent slide-to-slide variation
[#]: Slide unscorable

Table B.6.8.1.1.7.7-2: Results of the UDS assay with AMPA, second experiment ([redacted] 1991)

Second experiment			
	Net grains / nucleus Mean ± SE	Median	% of cells in repair
Medium control[#]	-12.6 ± 0.5	-13.5	2
Test item [µg/mL]			
5.00	-11.1 ± 0.9	-10.8	1
10.00	-12.7 ± 2.2	-12.8	2
50.00	-13.0 ± 1.9	-12.2	1
100.00	-12.3 ± 1.5	-12.2	1
250.00	-11.5 ± 1.3	-12.2	2
500.00	-11.8 ± 1.1	-12.2	2
1000.00	-11.6 ± 1.2	-11.5	2
2500.00	-8.8 ± 1.5	-8.8	1
3800.00	Toxic[#]	-	-
5000.00	Toxic[#]	-	-
Positive control 2-AAF [3 µg/mL]	19.7 ± 3.0	20.3	85

2-AAF: 2-acetylaminofluorene
SE: Standard errors, represent slide-to-slide variation
[#]: Slide unscorable

III. CONCLUSIONS

Based on the results of the present study, AMPA, metabolite of glyphosate did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In the present study, AMPA was negative for unscheduled DNA synthesis in primary rat hepatocytes *in vitro*. The study was conducted in accordance with GLP and similar to OECD guideline 482 (1986), which was deleted in 2014. The study was considered to provide supporting information only because the Unscheduled DNA Synthesis (UDS) assay is no longer a standard method described by current guidelines. There were no deviations when compared to the previous OECD guideline 482 (1986).

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion that, in this test, AMPA was negative for unscheduled DNA synthesis in primary rat hepatocytes *in vitro*. Considering that the UDS assay is no longer a standard method, the study was considered not acceptable though.
The study was accepted in the previous evaluation (RAR, 2015).

Study 8

Data point	KCA 5.8.1/045
Report author	[redacted]
Report year	2021
Report title	Aminomethylphosphonic acid (AMPA): Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*
Report No	8442150
Document No	CV-2020-0209
Guidelines followed in study	OECD 471 (1997), Commission Regulation (EC) no. 440/2008 Method B13/14 (2008), U.S. EPA OCSPP 870.5100 (1998), Japanese MAFF (2011), ICH S2 (R1, 2012)
Deviations from current test guideline OECD 471 (1997)	ENNG was used as positive control for strains TA 100 and TA 1535, and 4-NQO was use as positive control for strain TA 98. According to OECD 471, these compounds are only listed as positive controls for *E. coli* strains. Since the controls induced a consequent marked increase of revertants (refer to HCD of the positive controls), this deviation is accepted.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes/yes The study was conducted at Covance Laboratories Ltd., Shardlow, Derbyshire, United Kingdom
Acceptability/Reliability	**Conclusion GRG:** Yes/yes, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

Executive summary

Aminomethylphosphonic acid (AMPA, batch: 107785, purity: 99.2%) was assessed for its potential to induce gene mutations in bacteria. An Ames test was conducted with *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA in the presence and absence of metabolic activation (phenobarbitone/β-naphthoflavone-induced rat liver S9 fraction). Two independent experiments were performed using the plate-incorporation method (standard plate test, first experiment) and the pre-incubation method (second experiment). Bacteria were treated with the test item in a concentration range of 1.5 – 5000 µg/plate for both experiments. Negative (untreated), vehicle (sterile distilled water) and positive controls were included in each experiment. All incubations were performed in triplicates. After an incubation period between 48 and 72 hours, the plates were inspected for a possible reduction in the bacterial background lawn and the number of revertant colonies were counted for each plate.

There was no precipitation observed up to the highest tested concentration, neither in the presence nor in the absence of S9 mix. Cytotoxicity, evident as a reduction in the bacterial background lawn or in the reduction of spontaneous revertants when compared to vehicle controls, was not observed at any dose level, either in the presence or in the absence of metabolic activation.

In the first experiment, there were no statistically significant increases in the frequency of revertant colonies noted for any of the bacterial strains at any dose level, neither with nor without S9 mix. In the second experiment, there was a statistically significant increase in the number of revertant colonies observed for strain TA 100 at 500 µg/plate in the absence of S9 mix. The increase did not meet the acceptability criteria for a positive response and, in the lack of a dose-response relationship or reproducibility, was considered to be of no biological relevance.

The number of revertant colonies for the vehicle control and for negative (untreated) controls were considered acceptable. Although single counts were slightly outside the range of historical control data (TA 98 without S9 mix in the first experiment and TA 100 without S9 mix in the second experiment), the values were considered acceptable since the other control counts were within the expected range and the tested strains responded very well to the respective positive controls in both the presence and absence of S9 mix.

All of the positive controls induced marked increases in the frequency of revertant colonies, thus confirming the activity of the metabolic activation system and the sensitivity of the test itself.

Based on the experimental findings, the test item is not mutagenic in bacteria with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	White crystalline solid
Lot/Batch #:	107785
Purity:	99.2 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature in the dark and over silica gel) was guaranteed until 30 Sep 2021. The stability of the test item in solvent (vehicle) was verified by analytical methods. All formulations were shown to be stable and homogenous during the test performance.
Solvent (vehicle) used:	Sterile distilled water

2. Control materials:

Negative control:	Untreated controls were included in each experiment.
Solvent (vehicle) control:	Sterile distilled water
Solvent (vehicle)/final concentration:	0.1 mL/plate
Positive controls:	Please refer to table below.

Strain	**Metabolic activation**	**Mutagen**	**Solvent**	**Conc. [µg/plate]**
S. typhimurium strains				
TA 100	-S9	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	DMSO	3.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	1.0
TA 1535	-S9	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	DMSO	5.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.0
TA 98	-S9	4-Nitroquinoline-1-oxide (4-NQO)	DMSO	0.2
	+S9	Benzo[a]pyrene (BP)	DMSO	5.0
TA 1537	-S9	9-Aminoacrinidine (9AA)	DMSO	80.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.0
E. coli strain				
WP2 uvrA	-S9	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	DMSO	2.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	10.0

3. Metabolic activation:

S9 microsomal fraction was purchased from [redacted] (Lot No. 4222) and produced from livers of male Sprague-Dawley rats. The animals were 5-6 weeks of age and weighed 175 – 199 g. They were treated with phenobarbitone and β-naphthoflavone. A copy of the S9 Quality Control and Production Certificate is presented in the study report. The protein level was adjusted to 20 mg/mL. The S9 mix was prepared before use by adding sterilized co-factors and maintained on ice for the duration of the test. The S9 mix contained the following cofactors:

S9 mix component	Concentration	Unit
0.2 M Sodium phosphate buffer (pH 7.4)	25.0	mL
1.65 M KCl/0.4 M $MgCl_2$	1.0	mL
NADPH-generating system		
0.1 M Glucose 6-phosphate	2.5	mL
0.1 M NADP	2.0	mL
Sterile distilled water	14.5	mL
S9	5.0	mL

4. Test organisms:

Tester strains				**Bacteria batch checked for**	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

On a regular basis (approximately monthly), batches of culture from master stocks were prepared, coded, and then routinely tested for appropriate characteristics, viability and mutation frequency to ensure acceptability criteria were met.

5. Test concentrations:

Plate incorporation test ± S9 mix:	
Concentrations:	1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA
Replicates:	Triplicates
Pre-incubation test ± S9 mix:	
Concentrations:	1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA
Replicates:	Triplicates

B: STUDY DESIGN AND METHODS

8. Dates of experimental work: 03 Jun – 21 Sep 2020
Finalisation date: 25 Feb 2021

9. Standard plate test (plate-incorporation test, SPT):

An aliquot of 0.1 mL test solution, vehicle or positive control were added together with 0.1 mL bacterial strain culture, 0.5 mL of phosphate buffer (in tests without metabolic activation) or S9 mix (in tests with metabolic activation) and 2 mL of molten, trace amino-acid supplemented media (1.0 mM histidine + 1.0 mM biotin or 1.0 mM tryptophan). After mixing, the mixture was then overlayed onto a Vogel-Bonner agar plate. Negative (untreated) controls were included in each experiment. The plates were incubated at 37 ± 3 °C for 48 – 72 hours. Thereafter, all plates were scored for the presence of revertant colonies and viewed microscopically for evidence of thinning of the background bacterial lawn (toxicity).

10. Pre-incubation test (PIT):

0.1 mL of test solution, vehicle or positive control were mixed together with 0.1 mL of appropriate bacterial strain culture, 0.5 mL phosphate buffer (in tests without metabolic activation) or 0.5 mL of S9 mix (in tests with metabolic activation). The solution was pre-incubated for 20 minutes at 37 ± 3 °C while shaking prior to the addition of 2.0 mL of molten amino acid supplemented medium (1.0 mM histidine + 1.0 mM biotin or 1.0 mM tryptophan) and subsequent plating of the mixture onto Vogel-Bonner agar plates. Negative (untreated)

controls were included in each experiment. The plates were incubated at 37 ± 3 °C for 48 – 72 hours. Thereafter, all plates were scored for the presence of revertant colonies and viewed microscopically for evidence of thinning of the background bacterial lawn (toxicity).

11. Cytotoxicity

Toxicity was detected by
- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

12. Statistics

Statistical analysis was performed according to the UKEMS sub-committee on guidelines for mutagenicity testing[2]. Statistical significance was evaluated by using Dunnett's Regression Analysis ($p < 0.05$) for those values that indicate statistically significant increases in the frequency of revertant colonies compared to the concurrent solvent control.

13. Acceptability criteria

The test was considered valid if the following criteria were met:
- All bacterial strains must have demonstrated the required characteristics as determined by their respective strain checks.
- All tester strains cultures exhibited a characteristic number of spontaneous revertants per plate in the vehicle and untreated controls (7 – 40 for TA 1535, 60 – 200 for TA 100, 2 – 30 for TA 1537, 8 – 60 for TA 98 and 10 – 60 for WP2 uvrA). These values were confirmed by current in-house historical control data.
- All tester strain cultures were in the range of 0.9 – 9 x 10^9 bacteria per mL.
- Positive control chemicals induced marked increases in the frequency of revertant colonies, both with or without metabolic activation, which are in the range of historical control data.
- There was a minimum of 4 non-toxic test item concentrations.
- There was no evidence of excessive contamination.

14. Evaluation criteria

The test item was considered positive for mutagenicity if the following criteria were met:
- There was a dose-related increase in mutant frequency over the dose range tested.
- There was a reproducible increase at one or more concentrations.
- There was biological relevance against in-house historical control ranges.
- A fold increase greater than two times the concurrent solvent control for TA 100, TA 98 and WP2 uvrA or a three-fold increase for TA 1535 and TA 1537 (especially if accompanied by an out-of-historical range response).

[2] Mahon G.A.T. (1989) Analysis of data from microbial colony assays. In: KIRKLAND D.J. (eds). Statistical Evaluation of Mutagenicity Test Data. Cambridge University Press Report, pp. 26-65

A test item was considered non-mutagenic (negative) in the test system if the above-mentioned criteria were not met.

II. RESULTS AND DISCUSSION

E. ANALYTICAL DETERMINATIONS

Dose formulation analysis was carried out to determine the concentration of the test item. Results of formulation analyses showed that the tested concentrations were within 100 % ± 10 % of the nominal concentrations and that they were stable and homogenous during the test performance.

F. CYTOTOXICITY

In the first experiment (plate incorporation test), as well as in the second experiment (pre-incubation test), cytotoxicity, evident as a visible reduction in the growth of the bacterial background lawn or as a reduction in the number of spontaneous revertant colonies, was not observed at any dose level in the presence or in the absence of metabolic activation.

G. SOLUBILITY

Precipitation of the test item was not observed on the plates at any of the dose levels tested, neither in the presence nor in the absence of S9 mix.

H. MUTATION ASSAY

For the first experiment, there was no statistically significant increase in the frequency of revertant colonies recorded for any of the bacterial strains at any dose of the test item, either with or without metabolic activation.

For the second experiment, there was a statistically significant increase in the number of revertant colonies noted for TA 100 at 500 µg/plate in the absence of metabolic activation. However, the maximum fold increase was only 1.2 times the concurrent vehicle control, did not meet the acceptability criteria for a positive response and no reproducibility or dose-response relationship was observed. As no statistically significant changes were observed at any other dose level, this response was considered of no biological relevance.

The number of revertant colonies for the negative (untreated) controls and for the vehicle controls were considered acceptable. However, a single count for the vehicle control (TA 98 in the absence of S9-mix after the first mutation test) was just below the minimum level of the in-house historical vehicle control minima for the tested strain. In addition, two counts for the vehicle control (TA 100 dosed in the absence of S9-mix after the second mutation test) were just above the maximum level of the in-house historical vehicle control minima for the tested strain. All of these counts were considered acceptable since the other control counts were within the expected range and the tested strains responded very well to the respective positive controls in both the presence and absence of S9 mix.

All of the positive control chemicals induced marked increases in the frequency of revertant colonies, both with and without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9 mix were validated.

Table B.6.8.1.1.7.8-1: AMPA: Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*, first experiment ([redacted], 2021)

Experiment 1: Standard plate test (SPT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
water mean *± SD*	102 *± 12.2*	101 *± 4.6*	12 *± 1.5*	10 *± 4.4*	25 *± 4.5*	26 *± 5.7*	20 *± 6.7*	21 *± 6.2*	14 *± 3.5*	12 *± 1.0*
HCD[#] 2018, mean *± SD*	122 *± 18.8*	125 *± 21.5*	17 *± 4.2*	14 *± 3.1*	27 *± 5.3*	36 *± 6.2*	22 *± 4.5*	27 *± 5.1*	12 *± 3.3*	13 *± 3.2*
HCD[#] 2019, mean *± SD*	116 *± 18.1*	122 *± 18.8*	17 *± 4.8*	14 *± 3.6*	25 *± 5.9*	32 *± 6.7*	23 *± 5.4*	28 *± 5.9*	12 *± 3.4*	13 *± 3.3*

Table B.6.8.1.1.7.8-1: AMPA: Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*, first experiment ([redacted], 2021)

Experiment 1: Standard plate test (SPT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
2018/2019 [range]	*67 - 170*	*64 - 187*	*7 - 38*	*7 - 31*	*11 - 47*	*16 - 55*	*11 - 41*	*13 - 50*	*4 - 25*	*3 - 25*
Test item [µg/plate]										
1.5 mean *± SD*	101 *± 11.4*	110 *± 5.9*	10 *± 5.3*	11 *± 1.5*	23 *± 10.0*	32 *± 7.6*	17 *± 3.8*	24 *± 7.0*	12 *± 3.5*	12 *± 6.4*
5 mean *± SD*	82 *± 11.4*	87 *± 8.1*	15 *± 9.5*	13 *± 1.5*	21 *± 1.2*	26 *± 1.2*	17 *± 2.1*	25 *± 10.6*	17 *± 8.1*	14 *± 4.0*
15 mean *± SD*	88 *± 9.6*	97 *± 11.6*	13 *± 2.1*	8 *± 2.0*	27 *± 6.4*	31 *± 5.0*	20 *± 5.9*	26 *± 7.4*	10 *± 1.7*	12 *± 1.0*
50 mean *± SD*	86 *± 2.1*	89 *± 5.7*	12 *± 2.1*	14 *± 3.2*	26 *± 4.9*	29 *± 1.5*	23 *± 5.0*	20 *± 2.1*	13 *± 4.6*	11 *± 4.0*
150 mean *± SD*	102 *± 15.9*	99 *± 9.8*	14 *± 4.6*	11 *± 1.5*	24 *± 3.6*	28 *± 1.7*	23 *± 8.1*	29 *± 1.5*	13 *± 7.0*	11 *± 4.7*
500 mean *± SD*	94 *± 12.7*	92 *± 14.8*	10 *± 3.6*	12 *± 3.1*	24 *± 7.0*	30 *± 6.2*	20 *± 6.7*	24 *± 8.2*	8 *± 2.5*	10 *± 6.0*
1500 mean *± SD*	91 *± 6.6*	101 *± 5.5*	16 *± 2.1*	10 *± 8.7*	24 *± 4.2*	31 *± 4.0*	14 *± 2.1*	23 *± 5.5*	10 *± 6.2*	14 *± 1.2*
5000 mean *± SD*	92 *± 7.1*	78 *± 3.8*	12 *± 2.1*	12 *± 2.6*	27 *± 7.1*	32 *± 8.1*	21 *± 8.9*	24 *± 9.1*	9 *± 1.5*	11 *± 3.5*
Positive control										
[§] mean *± SD*	358 *± 39.0*	1703 *± 11.3*	112 *± 23.4*	251 *± 30.9*	379 *± 46.9*	205 *± 9.0*	143 *± 14.7*	109 *± 5.3*	401 *± 201.8*	242 *± 17.2*
HCD[#] mean 2018 *± SD*	606 *± 213.6*	1726 *± 528.7*	653 *± 484.4*	301 *± 57.2*	706 *± 235.8*	230 *± 74.8*	212 *± 77.1*	158 *± 49.3*	274 *± 150.4*	294 *± 86.8*
HCD[#] mean 2019 *± SD*	622 *± 294.0*	1381 *± 442.8*	790 *± 825.3*	268 *± 124.0*	629 *± 245.6*	175 *± 70.8*	186 *± 71.4*	165 *± 75.5*	266 *± 142.4*	232 *± 127.9*
2018/2019 [range]	*205 - 2322*	*318 - 3928*	*69 - 4595*	*112 - 1976*	*111 - 1420*	*99 - 790*	*92 - 477*	*79 - 719*	*76 - 833*	*109 - 1964*

[§] = information on respective positive control is reported in Material and Method section I.A.2

[#] = HCD of untreated and vehicle controls the values were combined. HCD consisting of >>200 values, expect for TA102 for which the HCD consists of 16-56 values per year.

Table B.6.8.1.1.7.8-2: AMPA: Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*, second experiment ([redacted], 2021)

Experiment 2: Pre-incubation Test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
water mean	155	126	30	16	31	34	19	27	10	8
± *SD*	± *19.9*	± *16.2*	± *4.2*	± *4.9*	± *4.0*	± *2.9*	± *3.5*	± *8.2*	± *1.5*	± *2.6*
HCD[#] mean 2018	116	122	17	14	25	32	23	28	12	13
± *SD*	± *18.1*	± *18.8*	± *4.8*	± *3.6*	± *5.9*	± *6.7*	± *5.4*	± *5.9*	± *3.4*	± *3.3*
HCD[#] mean 2019	116	122	17	14	25	32	23	28	12	13
± *SD*	± *18.1*	± *18.8*	± *4.8*	± *3.6*	± *5.9*	± *6.7*	± *5.4*	± *5.9*	± *3.4*	± *3.3*
2018/2019 [range]	*67 - 170*	*64 - 187*	*7 - 38*	*7 - 31*	*11 - 47*	*16 - 55*	*11 - 41*	*13 - 50*	*4 - 25*	*3 - 25*
Test item [µg/plate]										
1.5 mean	161	106	29	17	28	31	18	17	10	14
± *SD*	± *9.1*	± *7.0*	± *5.1*	± *5.5*	± *7.0*	± *7.2*	± *2.0*	± *2.3*	± *7.2*	± *2.5*
5 mean	159	127	31	15	25	31	18	25	10	10
± *SD*	± *10.3*	± *8.5*	± *2.5*	± *4.0*	± *2.1*	± *2.6*	± *3.6*	± *6.7*	± *3.5*	± *3.5*
15 mean	147	127	26	13	25	32	15	23	8	6
± *SD*	± *1.2*	± *11.7*	± *5.5*	± *2.9*	± *6.1*	± *7.2*	± *3.0*	± *1.7*	± *0.6*	± *5.0*
50 mean	163	132	29	16	29	33	16	28	10	11
± *SD*	± *4.5*	± *4.0*	± *3.5*	± *3.1*	± *10.4*	± *4.0*	± *1.2*	± *2.6*	± *0.6*	± *4.6*
150 mean	156	135	30	18	32	36	18	24	9	15
± *SD*	± *10.0*	± *12.5*	± *6.7*	± *5.2*	± *6.1*	± *6.0*	± *3.2*	± *4.0*	± *1.5*	± *2.1*
500 mean	**179***	117	27	21	35	33	17	26	10	9
± *SD*	± *9.5*	± *5.7*	± *2.5*	± *1.5*	± *3.5*	± *6.1*	± *7.1*	± *4.6*	± *2.6*	± *1.5*
1500 mean	154	117	32	20	25	35	23	24	10	10
± *SD*	± *10.2*	± *17.6*	± *3.1*	± *9.5*	± *5.5*	± *7.0*	± *5.3*	± *0.6*	± *4.0*	± *1.5*
5000 mean	160	119	25	21	25	41	20	26	9	7
± *SD*	± *1.5*	± *12.1*	± *3.5*	± *3.1*	± *5.3*	± *7.6*	± *2.6*	± *0.6*	± *4.0*	± *1.7*
Positive control										
[§] mean	357	1534	212	178	319	201	187	97	88	162
± *SD*	± *53.8*	± *158.7*	± *106.0*	± *36.7*	± *55.2*	± *15.7*	± *10.1*	± *3.6*	± *20.5*	± *15.0*
HCD[#] mean	622	1381	790	268	629	175	186	165	266	232
± *SD*	± *294.0*	± *442.8*	± *825.3*	± *124.0*	± *245.6*	± *70.8*	± *71.4*	± *75.5*	± *142.4*	± *127.9*
HCD[#] mean 2019	622	1381	790	268	629	175	186	165	266	232
± *SD*	± *294.0*	± *442.8*	± *825.3*	± *124.0*	± *245.6*	± *70.8*	± *71.4*	± *75.5*	± *142.4*	± *127.9*
2018/219 [range]	*205 - 2322*	*318 - 3928*	*69 - 4595*	*112 - 1976*	*111 - 1420*	*99 - 790*	*92 - 477*	*79 - 719*	*76 - 833*	*109 - 1964*

* = statistically significantly increased when compared to the vehicle control

[#] = HCD of untreated and vehicle controls the values were combined. HCD consisting of >>200 values, expect for TA102 for which the HCD consists of 16-56 values per year.

III. CONCLUSION

Under the conditions of the present study, the test item is not mutagenic in the Ames test (standard plate and pre-incubation method) with and without metabolic activation.

Assessment and conclusion by applicant:
In the present Reverse Mutation Assay (Ames Test) using strains of *S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) the test item did not induced an increase in the frequency of revertant colonies in the presence and in the absence of metabolic activation. Under the conditions of this test, the test item is considered to be non-mutagenic. The study was performed according to OECD guideline 471 (1997) and compliant with GLP. There were no deviations. Therefore, the study is considered valid and acceptable.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. AMPA did not induce a biologically relevant increase in the number of revertants under any of the test conditions. Under the conditions of this test, AMPA is not considered to be mutagenic with and without metabolic activation.
In contrast to the applicant's conclusion, however, a minor deviation from the OECD 471 recommendations was noticed (see above). This deviation is not expected to have an impact on the study outcome.

Study 9

Data point	5.8.1/046
Report author	[redacted]
Report year	2021
Report title	Aminomethylphosphonic acid (AMPA): V79 HPRT Gene Mutation Assay
Report No	8441963 (Covance Laboratories Ltd.)
Document No	CV-2020-0233
Guidelines followed in study	OECD 476 (2016), Commission Regulation (EC) No. 440/2008 method B17 (2008), US EPA OPPTS 870.5300 (1998)
Deviations from current test guideline OECD 476 (2016)	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes/Yes The study was performed at Covance Laboratories Limited, Shardlow, UK
Acceptability/Reliability	**Conclusion GRG:** Yes/Yes, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

Executive summary

Aminomethylphosphonic acid (batch: 107785, purity: 99.2%) was tested *in vitro* for its mutagenic potential on the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus of the V79 cell line in the presence and absence of a metabolic activation system. Test item concentrations were selected based on the results of a preliminary cytotoxicity test where the results indicated that the maximum concentration should be the 10 mM limit concentration. For the main test, concentrations of test item used were 0, 34.69, 69.38, 138.75, 277.5, 555, 1110 µg/mL in both the absence and presence of metabolic activation. Cells were exposed for 4 hours and analysed for relative survival, cloning efficiency, and expression of mutant colonies.

Following exposure with and without S9 mix, the cells were re-plated and incubated for 7 days to allow expression of the mutant phenotype. At the end of the expression period the cell monolayers were detached using trypsin, cell suspensions counted, and plated out either to determine cloning efficiency or mutant frequency. Determining mutant frequency, dishes were incubated for 7 days supplemented with 6-Thioguanine (6-TG).

At the end of the exposure period, no precipitate of the test item was observed at any of the dose levels, in either the absence or presence of metabolic activation.

The test item demonstrated no statistically significant increases in mutant frequency at any of the dose levels in the absence of metabolic activation. In the presence of metabolic activation, a small but statistically significant increase in mutant frequency was observed at 34.69 µg/mL and there was also evidence of a very modest statistically significant concentration related increase. However, the mutant frequency values observed would have been considered acceptable for vehicle controls and all the values at dose levels where the mutant

frequency exceeded the vehicle control were within 95% control limits for vehicle controls. The responses were therefore considered to be spurious and of no toxicological significance.

Based on the experimental findings and under the conditions of the test, there was no evidence for gene mutation in mammalian cells *in vitro*, neither in the presence nor in the absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	White crystalline solid
Lot/Batch number:	107785
Purity:	99.2% (no purity correction required)
Stability of test compound:	The stability of the test item at storage conditions was guaranteed until the expiry date 30 Sep 2021. The stability of the test substance in vehicle was not specified.

2. Control material:

Negative control:	The negative control was actually the solvent control.
Solvent (vehicle) control:	Water
Positive control:	- S9 mix: Ethylmethane sulfonate (EMS), 500 and 750 µg/mL in DMSO + S9 mix: 9,10-Dimethyl-1,2-benzanthracene (DMBA), 1 and 2 µg/mL in DMSO

3. Metabolic activation:

The S9 Microsomal fraction used during the course of the study was purchased from [redacted], Lot No. 4127, Expiry February 2021, and Lot No. 4222, Expiry 12 March 2022. Copies of the Quality Control & Production Certificates, demonstrating the capability to activate known mutagens, are presented in the study report. The protein content was adjusted to 20 mg/ml prior to use The S9 mix was prepared by mixing S9 co-factors as follows:

S9 mix component	Concentration	Unit
Phosphate buffer containing NADP	5	mM
Glucose 6-phosphate	5	mM
KCL	33	mM
$MgCl_2$	8	mM
S9	2	%

The final concentration of S9 when dosed at a 10% volume of S9-mix was 2% for the Preliminary Toxicity Test and the Main Experiment.

4. Test organism:

The Chinese hamster V79 cell stocks were obtained from Harlan CCR in 2010 and originated from Labor für Mutagenitätsprüfungen (LMP); Technical University; 64287 Darmstadt, Germany.

5. Cell culture media:

Cultivation medium (MEM-FBS):	Eagles Medium (MEM) supplemented with sodium bicarbonate, L-glutamine, penicillin/streptomycin, amphotericin B, HEPES buffer and 10% fetal bovine serum (FBS)
Treatment medium (± S9):	Serum-free Minimal Essential Medium (MEM)
Selection medium:	Cultivation medium (MEM-FBS) supplemented with 11 µg/mL 6-thioguanidine (6TG)
Incubation:	At 37 °C in a humidified atmosphere containing 5% CO_2

6. Locus examined: Hypoxanthine-guanine phosphoribosyl transferase (HPRT)

7. Test concentrations and number of replicates:

(a) Preliminary cytotoxicity test

Metabolic activation	Duration of exposure	Concentrations (µg/mL)	Replicates
- S9 mix	4 h	4.34, 8.67, 17.34, 34.69, 69.38, 138.75, 277.5, 555, 1110	Single culture
+ S9 mix	4 h	4.34, 8.67, 17.34, 34.69, 69.38, 138.75, 277.5, 555, 1110	Single culture

(b) Mutagenicity test – Main experiment

Metabolic activation	Duration of exposure	Concentrations (µg/mL)	Replicates
- S9 mix	4 h	34.69*, 69.38*, 138.75*, 277.5*, 555*, 1110*, EMS 500* and EMS 750*	Duplicate
+ S9 mix	4 h	34.69*, 69.38*, 138.75*, 277.5*, 555*, 1110*, DMBA 1.0* and DMBA 2.0*	Duplicate

* = Dose levels plated out for cloning efficiency and mutant frequency
EMS = Ethyl methane sulphonate
DMBA = Dimethyl benzanthracene

B. STUDY DESIGN AND METHODS

2. Dates of experimental work: 02 Jun – 01 Oct 2020

Finalisation date: 25 Feb 2021

2. Preliminary cytotoxicity test:

A preliminary test was performed to identify suitable dose levels for the main mutagenicity study. Cell cultures were established identically to the performance in the main mutagenicity test described below. Cells were exposed to 4.34, 8.67, 17.34, 34.69, 69.38, 138.75, 277.5, 555, 1110 µg/mL for 4 hours in the presence and absence of S9 mix. For each condition, three replicate plates were used. Following exposure, the cells were washed twice with phosphate buffered saline (PBS), trypsinised and plated to determine a relative survival. After 7 days of incubation at 37 °C, cells were fixed, stained and counted.

Based on the results of the preliminary test, the test item concentrations for the main mutagenicity test were selected.

3. Main mutation assay:

Pre-treatment of cells:

For each condition, 2 x 10^6 cells were seeded into 225 cm² flasks and incubated at 37 °C. This was demonstrated to provide at least 20 x 10^6 available for dosing in each flask using a parallel flask.

Treatment:

Duplicate cultures were set up, both in the presence and absence of metabolic activation, with six test item concentrations, and vehicle and positive controls. Treatment was for 4 hours in serum free media (MEM) at approximately 37 °C in an incubator with a humidified atmosphere of 5% CO_2 in air. The concentrations of test item used was 0, 34.69, 69.38, 138.75, 277.5, 555, 1110 µg/mL in both the absence and presence of metabolic activation.

Samples of all the dose formulations were taken for dose formulation analysis.

Expression/Selection period:

During the 7-Day expression period the cultures were sub-cultured and maintained on days 2 and 5 to maintain logarithmic growth. At the end of the expression period, the cell monolayers were detached using trypsin, cell suspensions counted using a Coulter counter and plated out as follows:

i) In triplicate to determine cloning efficiency. Flasks were incubated for 6 days, fixed with methanol and stained with Giemsa.

ii) In petri dishes (ten replicates per group) supplemented with 11 µg/mL 6-Thioguanine (6-TG), to determine mutant frequency. Dishes were incubated for 7 days, then fixed with methanol and stained with Giemsa (Selection period).

4. Cytotoxicity:

Day 0 viability

The Day 0 viability of test item-treated cells relative to solvent controls was determined in parallel to the mutagenicity test. At the end of the exposure period, a sample of each cell culture was collected to assess survival of the cells. Triplicates of 200 cells/25 cm² dish were seeded and incubated for 6 days. Afterwards, the colonies were fixed, stained and counted. Based on the Day 0 viability, the relative survival (RS) was calculated.

Day 7 viability

The Day 7 viability was determined in parallel to the selection of mutants. After the expression period, triplicates of 200 cells/25 cm² dish were seeded in medium without 6-thioguanine to assess cell viability. After an incubation period of 7 days, the colonies were fixed, stained and counted.

5. Evaluation and calculations:

Determination of cytotoxicity

Cloning efficiency (CE, termed Day x viability in the study report, %):

$$CE\% = \frac{\text{Mean number of colonies in the test group}}{\text{Total number of seeded cells in the test group (200)}} \times 100$$

Adjusted CE: $$\text{Adjusted CE} = CE\% \times \frac{\text{Number of cells at the end of treatment}}{\text{Number of cells at the beginning of treatment}}$$

Relative survival (RS, % of control): $$RS = \frac{\text{Adjusted CE in treated culture}}{\text{Adjusted CE in the solvent control}} \times 100$$

Determination of mutant frequency

The mutant frequency is defined by the cloning efficiency of mutant colonies in selective medium divided by the cloning efficiency in non-selective medium measured for the same culture at the time of selection was calculated for each sample.

The mutant frequency (MF) for each dose was calculated as follows:

$$MF = \frac{\text{Total number of mutant colonies}}{2}$$

The mutation frequency/10^{-6} survival rate (MFS 10^{-6}) was calculated regarding the values of CE_2:

$$MFS\ 10^{-6} = \frac{MF}{\text{Day 7 CE\%}} \times 100$$

6. Statistics:

Statistical analysis was performed, if there was an increase of mutant frequencies in any dose level. In this case, comparisons were made between the solvent control value and each individual dose level, using

Student's t-test. To assess the dose-relationship, a linear regression model was used. An arcsin square-root transformation was applied to the mutant frequency per survivor (excluding positive controls). A linear regression model was then applied to these transformed values with dose values fitted as the explanatory variable. The F-value from the model was assessed at the 5% statistical significance level.

7. Acceptance criteria:

The test was considered valid if the following criteria were met:

- The spontaneous mutant frequency of the solvent control was within the range of the laboratory's historical control data.
- The positive controls caused an increase in mutation frequency comparable to the historical control data and statistically significantly higher than the solvent control.
- The criteria for selection of the maximum concentration as specified in the guideline have been met.
- Two experimental conditions (with and without metabolic activation) were tested unless one resulted in a positive response.
- Adequate numbers of cells and concentrations were analysable.
- A minimum of 4 analysed duplicate dose levels was considered necessary in order to accept a single assay for evaluation of the test item.

8. Evaluation criteria:

A test item was judged positive for gene mutation in mammalian cells if the following criteria were met:

- A significant increase in mutant frequency was observed in at least one concentration when compared to the solvent control.
- The increase was concentration dependent.
- The results were outside the range of the historical solvent control data for the test item concentrations.

A test item was judged negative for gene mutation in mammalian cells if the following criteria were met:

- The test item did not increase the mutation frequency compared to the solvent control under any condition.
- There was no concentration dependent increase.
- The results of the test item concentration were within the range of the historical solvent control data.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS

The dose formulation analysis performed for the main experiment demonstrated that the test item formulations were accurate and within acceptable limits. The analysed concentrations ranged from 99 – 104% (after 4 hours of storage) and 95 – 101% (after 24 hours of storage) of the initial concentration. All mixtures were homogenous.

2. CYTOTOXICITY

In the preliminary cytotoxicity test, there were no marked concentration-related reductions in the relative survival values in either the absence or presence of metabolic activation. Based on these findings, the highest test item concentrations for the main mutagenicity study was 10 mM.

In the main test, no cytotoxicity was observed. Neither RS nor CE_2 were affected by treatment with AMPA at any test item concentration with and without metabolic activation.

3. SOLUBILITY

In the preliminary cytotoxicity test as well as in the main mutation test and, no precipitate of the test item was observed at any of the dose levels, in either the absence or presence of metabolic activation.

There was no significant change in pH when the test item was dosed into media and the osmolality did not increase by more than 50 mOsm at the concentration levels investigated. The pH and osmolality readings from the solubility check are in the following table:

Concentration (µg/mL)	0	4.34	8.67	17.34	34.69	69.38	138.75	277.5	555	1110
pH	7.25	7.26	7.29	7.31	7.31	7.30	7.28	7.20	7.06	6.82
Osmolality mOsm	282	284	286	283	282	281	284	284	285	287

4. MUTANT FREQUENCY

The test item demonstrated no statistically significant increases in mutant frequency at any of the dose levels in the absence of metabolic activation. There were also no statistically significant concentration related increases in the absence of metabolic activation when evaluated with a trend test.

In the presence of metabolic activation, a small but statistically significant increase in mutant frequency was observed at the lowest dose tested of 34.69 µg/mL. According to the study authors there was also evidence of a very modest statistically significant concentration related increase, however, no further details were provided (no p-value given). According to the RMS, based on the mutant frequencies reported in the table below no clear trend is visible (9, 14, 5, 11, 11, 11 and 6 MFS (10^{-6}) for the test concentrations of 0, 34.69, 69.38, 138.75, 277.5, 555 and 1110 µg/mL, respectively). As the mutant frequency values observed would have been considered acceptable for vehicle controls and all the values at dose levels where the mutant frequency exceeded the vehicle control were within 95% control limits for vehicle controls, the responses were considered to be spurious and of no toxicological significance.

Mutant frequencies of the solvent control cultures remained within the range of the laboratories historical control data. The positive control mutagens EMS and DMBA showed the expected results, thereby demonstrating the functionality of the S9 mix and the sensitivity of the test.

Table B.6.8.1.1.7.9-1: Results of the HPRT gene mutation assay in mammalian cells with AMPA ([redacted] 2021): 4-hour incubation without metabolic activation

Concentration (µg/mL)		Day 0 viability				Day 7 viability			Day 7 Mutant frequency			
		% CE	CE	RS	**Mean RS**	% CE	% Control	**Mean % control**	MF	MFS 10^{-6}	SD	**Group MFS 10^{-6}**
Vehicle (Water)												
0	A	85.7	84.9	100	**100**	84.3	100	**100**	9.5	11.3	1.47	**16**
	B	77.7	91.5	100		75.7	100		15	19.8		
HCD mean ± SD										13.08 ± 3.45		
range										8 - 20		
Test item												
34.69	A	90.5	89.7	105.6	**105**	82.3	97.6	**106**	15.5	18.8	1.92	**18**
	B	81.2	95.7	104.5		87.0	115.0		14.5	16.7		
69.38	A	77.3	76.7	90.3	**100**	63.3	75.1	**97**	8	12.6	1.52	**14**
	B	84.8	100.0	109.2		90.3	119.4		14.5	16.1		
138.75	A	77.7	77.0	90.7	**91**	89.3	105.9	**110**	12.5	14.0	1.73	**15**
	B	70.2	82.7	90.3		85.7	113.2		14	16.3		
277.5	A	80.7	80.0	94.2	**92**	82.5	97.8	**102**	15.5	18.8	1.86	**18**
	B	69.8	82.3	89.9		80.3	106.2		13.5	16.8		
555	A	79.7	79.0	93.0	**98**	80.3	95.3	**101**	12.5	15.6	0.68	**15**
	B	80.7	95.1	103.9		80.3	106.2		11.5	14.3		
1110	A	84.5	83.8	98.6	**102**	75.7	89.7	**94**	7	9.3	1.10	**9**
	B	81.7	96.3	105.2		75.0	99.1		7	9.3		
Positive control (EMS)												
500	A	53.0	52.5	61.9	**75**	71.0	84.2	**90**	253	356.3	10.46	**298*** **
	B	68.8	81.1	88.6		73.2	96.7		175.5	239.9		
HCD mean ± SD										281.91 ± 86.04		
range										133 - 574		
750	A	33.3	33.0	38.9	**41**	60.7	71.9	**93**	373.5	615.7	11.04	**548*** **
	B	33.2	39.1	42.7		86.3	114.1		414	479.5		
HCD mean ± SD										454.65 ± 154.25		
range										169 - 799		

EMS: Ethylmethane sulfonate; CE: Cloning efficiency; RS: relative survival; MF: Mutant frequency; MFS: Mutant frequency per survivor; statistical significance at *** $p < 0.001$ level; HCD: historical control data generated in the testing laboratory (time frame not specified), as available from the study report (n = 24 and n = 23 for the negative and positive HCD, respectively)

Table B.6.8.1.1.7.9-2: Results of the HPRT gene mutation assay in mammalian cells with AMPA ([redacted], 2021): 4-hour incubation with metabolic activation

Concentration (µg/mL)		Day 0 viability				Day 7 viability			Day 7 Mutant frequency			
		% CE	CE	RS	**Mean RS**	% CE	% Control	**Mean % control**	MF	MFS 10^{-6}	SD	**Group MFS 10^{-6}**
Vehicle (Water)												
0	A	81.7	74.4	100	**100**	96.7	100	**100**	10	10.3	1.10	**9**
	B	93.2	29.2	100		89.7	100		6	6.7		
HCD mean ± SD										12.54 ± 2.98		
range										8 - 19		
Test item												
34.69	A	81.0	73.8	99.2	**97**	91.2	94.3	**100**	12	13.2	1.22	**14****
	B	88.5	27.8	95.0		95.5	106.5		15	15.7		
69.38	A	82.2	74.8	100.6	**95**	89.8	92.9	**98**	4	4.5	0.99	**5**
	B	82.7	26.0	88.7		91.8	102.4		4.5	4.9		
138.75	A	70.0	63.8	85.7	**91**	77.0	79.7	**93**	7	9.1	1.04	**11**
	B	90.5	28.4	97.1		94.8	105.8		11.5	12.1		
277.5	A	81.0	73.8	99.2	**94**	90.0	93.1	**93**	5.5	6.1	1.14	**11**
	B	83.5	26.2	89.6		82.5	92.0		13	15.8		
555	A	89.7	81.7	109.8	**103**	84.2	87.1	**89**	9	10.7	0.95	**11**
	B	89.8	28.2	96.4		81.0	90.3		9	11.1		
1110	A	73.0	66.5	89.4	**95**	82.8	85.7	**93**	6.5	7.8	1.00	**6**
	B	94.0	29.5	100.9		90.3	100.7		3	3.3		
Positive control (DMBA)												
1	A	65.5	59.7	80.2	**86**	94.0	97.2	**92**	243	258.5	6.61	**300*****
	B	85.5	26.8	91.8		77.8	86.8		265.5	341.1		
HCD mean ± SD										362.26 ± 107.26		
range										116 - 540		
2	A	66.0	60.1	80.8	**84**	82.8	85.7	**79**	333	402.0	7.18	**461*****
	B	82.0	25.7	88.0		65.0	72.5		338.5	520.8		
HCD mean ± SD										601.22 ± 227.95		
range										265 - 1320		

DMBA: Dimethyl benzanthracene; CE: Cloning efficiency; RS: relative survival; MF: Mutant frequency; MFS: Mutant frequency per survivor; statistical significance at ** $p < 0.01$ and *** $p < 0.001$ level; HCD: historical control data generated in the testing laboratory (time frame not specified), as available from the study report (n = 24 and n = 23 for the negative and positive HCD, respectively)

III. CONCLUSIONS

Based on the experimental findings and under the conditions of the test, AMPA did not induce any toxicologically significant or dose-related increase of gene mutations in the HPRT locus of V79 cells, neither in the presence nor in the absence of metabolic activation. It is therefore considered negative for mutagenicity in mammalian cells *in vitro*.

Assessment and conclusion by applicant:
Under the conditions of the test, Aminomethylphosphonic acid (AMPA) was negative for mutagenicity at the HPRT locus in V79 cells with and without metabolic activation.

The study was conducted in compliance with GLP and according to OECD guideline 476 (2016). The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. AMPA (batch 107785, purity: 99.2%)) was negative for gene mutation at the HPRT gene locus of V79 cells with and without metabolic activation under the conditions of this *in vitro* cell gene mutation assay.

Study 10

Data point	5.8.1/047
Report author	[redacted]
Report year	2021
Report title	Aminomethylphosphonic acid (AMPA): Micronucleus Test in Human Lymphocytes *in vitro*
Report No	8442149
Document No	CV-2020-0208
Guidelines followed in study	OECD 487 (2016)
Deviations from current test guideline OECD 487 (2016)	Modification of the suggested extended treatment schedule (see study summary). The RMS agrees with the justification for this modification.
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	Yes/yes The study was conducted at Covance Laboratories Ltd., Shardlow, Derbyshire, United Kingdom
Acceptability/Reliability	**Conclusion GRG:** Yes/yes, Category 1 **Conclusion AGG:** The study is considered to be acceptable

Executive summary

An *in vitro* study for the detection of the clastogenic and aneugenic potential of aminomethylphosphonic acid (AMPA, batch: 107785, purity: 99.2%) on the nuclei of normal human lymphocytes was performed in the presence and absence of metabolic activation (phenobarbital and 5,6-benzoflavone-induced rat liver S9 fraction). Based on the results of a preliminary cytotoxicity test, dose levels for the main micronucles test were in the range of 34.69 – 1110 µg/mL (corresponding to the limit test concentration of 10 mM).

Duplicate cultures were exposed for 4 hours in the absence or presence of S9 mix and for 24 hours in the absence of S9 mix. Solvent (water) and clastogenic (0.2 µg/mL mitomycin C for 4 hour exposure without S9 mix, 6 µg/mL cyclophosphamide for 4 hour exposure with S9 mix) and aneugenic positive controls (0.075 µg/mL demecolcine for 24 hour exposure without S9 mix) were included. After exposure, cells were cultivated in cytochalasin B-enriched medium for 24 hours prior to harvest.

A total of at least 2000 binucleated cells (4000 for solvent controls) were scored for the presence of micronuclei. In addition, cytotoxicity was evaluated as cytokinesis block proliferation index (CBPI).

There was no precipitation of the test substance in culture medium observed at the end of treatment, not for any concentration, neither with nor without S9 mix.

There was no marked cytotoxicity after 4 hours of exposure with or without S9 mix and after 24 hours of exposure without S9 mix.

The test item did not induce any statistically significant increases in the frequency of binucleate cells with micronuclei in any of the three exposure groups in the absence or presence of S9 mix. All results were within the distribution of the historical vehicle data (within 95% control limits) and there was also no statistically significant concentration related increase in any of the three exposure groups when evaluated with a trend test.

In both experiments, the frequency of micronucleated cells was within the range of the laboratory's historical control data for the solvent control. The clastogenic and aneugenic positive controls induced statistically significant increases in the frequency of micronucleated cells in all experiments, demonstrating the functionality of the metabolic activation system and the validity of the assay.

In conclusion, AMPA was considered to be non-clastogenic and non-aneugenic to human lymphocytes *in vitro*, in the presence and in the absence of metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: 1-aminomethylphosphonic acid (CAS No. 1066-51-9)

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	White crystalline solid
Lot/Batch #:	107785
Purity:	99.2% w/w
Stability of test compound:	The stability of the test item under storage conditions (at room temperature in the dark over silica gel) was guaranteed until 30 Sep 2021. The stability of the test item in solvent (vehicle) was verified by analytical methods.
Solvent (vehicle) used:	Water

2. Control materials

Negative control:	The solvent control is actually the negative control.
Solvent (vehicle) control:	Water
Positive controls:	Please refer to table below.

Clastogenic positive controls	
Short-term treatment (4-hour exposure)	
- S9	Mitomycin C (MMC), 0.2 µg/mL in Minimal essential medium
+ S9	Cyclophosphamide (CP): 6 µg/mL in DMSO
Aneugenic positive controls	
Long-term treatment (24-hour exposure)	
- S9	Demecolcine (DC), 0.075 µg/mL in sterile distilled water

Demecolcine (DC) is not one of the suggested positive control substances listed in the OECD 487 guideline but the substances are recommendations only, and DC is a derivative of Colchicine, one of the recommended substances. There is sufficient laboratory historical control data to demonstrate its effectiveness and suitability as an aneugen.

3. Metabolic activation:

The S9 Microsomal Enzyme Fraction was purchased from [redacted] (Lot no 4222, expiry date 12 March 2022) and obtained from the livers of male Sprague-Dawley rats, that were induced with phenobarbital and 5,6-

benzoflavone. The animals were 5-6 weeks of age and 175 – 199 g of weight. The protein content was adjusted to 20 mg/mL prior to use. The S9 was pre-tested for acceptability by the supplier prior to purchase and was supplied with a relevant "Quality Control & Production Certificate" which is presented in the study report.

Prior to each experiment, co-factors were added to the S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	5	mM
$MgCl_2$	8	mM
S9	20	%

The final concentration of S9 the cell culture medium was 2%.

4. Test organism:

For each experiment, sufficient whole blood was drawn from the peripheral circulation of a non-smoking volunteer (female, 35 years of age for the preliminary toxicity test and female, 24 years of age for main experiment) who had been previously screened for suitability. The cells were reported to have an average generation time of approximately 16 hours.

5. Cell culture:

Complete culture and treatment medium: Eagles minimal essential medium with HEPES (MEM) supplemented with 10% foetal bovine serum (FBS), penicillin/ streptomycin solution, L-glutamine and amphotericin B.

Incubation: At 37 °C and 5% CO_2 in humidified air.

Cell culture establishment prior to exposure

An amount of 0.5 – 0.73 mL of heparinised whole blood was cultured with 8.07 – 9.29 mL MEM supplemented with 10% FBS, 0.1 mL lithium heparin and 0.1 mL phytohaemagglutinin (PHA) for 48 hours prior to treatment.

6. Test concentrations and number of replicates:

a) Preliminary toxicity test

Metabolic activation	Duration of exposure (Fixation)	Concentrations	Replicates
± S9 mix	4 h (28 h)	4.34, 8.67, 17.34, 34.69, 69.38, 138.75, 277.5, 555, and 1110 µg/mL	Single culture (duplicate for control)
-S9 mix	24 h (48 h)	4.34, 8.67, 17.34, 34.69, 69.38, 138.75, 277.5, 555, and 1110 µg/mL	Single culture (duplicate for control)

b) Main micronucleus test

Metabolic activation	Duration of exposure (Fixation)	Concentrations	Replicates
± S9 mix	4 h (28 h)	34.69, 69.38, 138.75, 277.5*, 555*, and 1110* µg/mL	Duplicate (4 replicates for control)
- S9 mix	24 h (48 h)	34.69, 69.38, 138.75, 277.5*, 555*, and 1110* µg/mL	Duplicate (4 replicates for control)

*: Concentrations selected for microscopic analysis of micronucleated frequencies.

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 02 Jun – 22 Sep 2020
Finalisation date: 25 Feb 2021

2. Preliminary cytotoxicity test:

In a preliminary cytotoxicity test, human lymphocytes were treated with the test item at concentrations of 4.34 to 1110 µg/mL both, with and without metabolic activation under the same conditions as in the main mutagenicity test (described below). The maximum dose was the maximum recommended dose level by the guideline, equivalent to 10 mM concentration. One single cell culture per condition was exposed to the test item for 4 hours in the presence and absence of S9 mix or for 24 hours in the absence of S9 mix. Cells were prepared about 24 hours following the completion of exposure.

Using a qualitative microscopic evaluation of the microscope slide preparations from each treatment culture, appropriate dose levels were selected for the evaluation of the frequency of binucleate cells and to calculate the cytokinesis block proliferation index (CBPI). The CBPI data were used to estimate test item toxicity and for selection of the dose levels for the exposure groups of the main experiment.

3. Micronucleus test:

Treatment and cytokinesis block:

About 48 hours after cell culture establishment, approximately 9 mL of the culture medium was removed and replaced by fresh culture medium. 1 mL of the appropriate test item, vehicle or positive control solution was added.
Duplicate cultures were exposed for 4 hours in the absence or presence of S9 mix and for 24 hours in the absence of S9 mix at test item concentrations in the range of 34.69 - 1110 µg/mL. Solvent (water) and positive controls (0.2 µg/mL mitomycin C for 4 hour exposure without S9 mix, 6 µg/mL cyclophosphamide for 4 hour exposure with S9 mix and 0.075 µg/mL demecolcine for 24 hour exposure without S9 mix) were included.
Following exposure, cells were centrifuged and washed in fresh culture medium. Thereafter, cells were incubated in fresh culture medium including 4.5 µg/mL cytochalasin B for another 24 hours.

The RMS notes the following: The extended exposure for the extended treatment (24 hours without S9 mix) detailed above is a modification of the suggested cell treatment schedule in the OECD Guideline 487. According to the study director, this is considered to be an acceptable alternative because it avoids any potential interaction between cytochalasin B and the test item during exposure and any effect this may have on the activity or response. Additionally, the study directed stated that as the stability or reactivity of the test item is unknown prior to the start of the study this modification of the schedule is considered more effective and reproducible by the study director due to the in-house observations on human lymphocytes and their particular growth characteristics in this study type and also the significant laboratory historical control data using the above format. The RMS agrees with this justification on the modification of the suggested cell treatment schedule.

Cell harvest: At the end of the incubation with Cytochalasin B, the cells were centrifuged, the culture medium was drawn off and discarded, and cells were re-suspended in MEM. Cells were then treated with a mild hypotonic solution (0.0375 M KCl) before being fixed with fresh methanol/glacial acetic acid (19:1 v/v). The fixative was changed at least three times and the cells stored at approximately 4 ºC prior to slide preparation.

Slide preparation: Lymphocytes were re-suspended in several mL of fresh fixative before centrifugation and re-suspension in a small amount of fixative. Several drops of this suspension were dropped onto clean, wet microscope slides and left to air dry with gentle warming. Afterwards, cells were stained with 5% Giemsa.

Metaphase analysis A total number of at least 2000 binucleated cells per condition (1000 binucleated cells per culture) were examined by microscopy and scored for the presence of micronuclei. For the vehicle controls, a total of 4000 binucleated cells were scored.

Cytotoxicity: The cytokinesis block proliferation index (CBPI) was determined from 500 cells. In addition, the percentage of cytostasis was determined, which indicates the inhibition of cell growth in treated cultures in comparison to control cultures. Based on the current OECD guideline 487 (2016), cytotoxicity should not exceed the limit of 55 ± 5%.

Formulas: Calculation of the Cytokinesis Block Proliferation Index (CBPI):

$$CBPI = \frac{(c1\ x\ 1) + (c2\ x\ 2) + (cm\ x\ 3)}{n}$$

c_1 : mononucleate cells
c_2: binucleate cells
c_m: multinucleate cells
n: total number of cells

Calculation of cytostasis:

$$\%\ Cytostasis = 100 - 100\ x \frac{(CBPI\ treated\ culture - 1)}{(CBPI\ control\ culture - 1)}$$

4. Statistics:

Statistical significance at the 5% level ($p < 0.05$) was evaluated by the non-parametric Chi-square test. The p-value was used as a limit in judging for significance levels in comparison with the corresponding negative control. The dose-relationship (trend-test) was assessed using a linear regression model. An arcsin square-root transformation was applied to the percentage of binucleated cells containing micronuclei (excluding positive controls). A linear regression model was then applied to these transformed values with dose values fitted as the explanatory variable. The F-value from the model was assessed at the 5% statistical significance level.

5. Acceptability criteria:

The study was considered valid if the following criteria were met:

- The concurrent negative control was considered acceptable for addition to the laboratory historical negative control data range.
- All the positive control chemicals induced positive responses that were compatible with those in the laboratory historical positive control data range and produced a statistically significant increase when compared with the concurrent negative control. Acceptable positive responses demonstrated the validity of the experiment and the integrity of the S9-mix.
- Cell proliferation criteria in the solvent control were considered to be acceptable.
- The study was performed using all three exposure conditions using a top concentration which meets the requirements of the current testing guideline.
- The required number of cells and concentrations were analysed.

6. Evaluation criteria:

A test substance was considered positive for induction of micronuclei when the following criteria were met:

- At least one of the test concentrations exhibited a statistically significant increase compared with the concurrent negative control.
- The increase was dose-related in at least one experimental condition when evaluated with an appropriate trend test.
- The results were substantially outside the range of the laboratory historical negative control data.

A test item was considered negative for induction of micronuclei when the following criteria were met:

- None of the test concentrations exhibited a statistically significant increase compared with the concurrent negative control.
- There was no dose-related increase when evaluated with an appropriate trend test.
- The results in all evaluated dose groups were within the range of the vehicle laboratory historical control data.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The dose formulation analysis performed for the main experiment demonstrated that the test item formulations were accurate and within acceptable limits. (97 - 105% of the nominal concentrations). Stability and homogeneity were evaluated as part of a different study (method development study report no. 8442132; KCA 4.1.2-225). The test item formulations were shown to be stable for up to 24-hours.

B. CYTOTOXICITY

In the preliminary cytotoxicity test, there was no remarkable cytotoxicity observed in any of the three exposure groups at any dose level. The percentage of cytostasis at the highest dose level was 29 and 29%% after 4 hours exposure with and without S9 mix and 3% after 24 hours exposure without S9 mix.

In the main micronucleus test, no cytotoxicity was observed. The percentage of cytostasis was < 10% for all concentrations in all three exposure groups.

There was further no significant change in pH and no change on osmolality by more than 50 mOsm at any dose level tested.

C. SOLUBILITY

No test item precipitate was observed in the blood-free cultures at the end of the exposure period, neither with nor without S9 mix.

D. MICRONUCLEUS TEST

The test item did not induce any statistically significant increases in the frequency of binucleate cells with micronuclei in any of the three exposure groups in the absence or presence of S9 mix. All results were within the distribution of the historical vehicle data (within 95% control limits) and there was also no statistically significant concentration related increase in any of the three exposure groups when evaluated with a trend test.

Vehicle control cultures induced the expected range of frequencies of cells with micronuclei and were considered valid and acceptable. The clastogenic and aneugenic positive controls induced distinct and statistically significant increases in the frequency of micronucleated cells in all experiments, demonstrating the functionality of the metabolic activation system and the validity of the assay.

Table B.6.8.1.1.7.10-1: AMPA - Summary of genotoxicity data obtained in the micronucleus test in human lymphocytes *in vitro* ([redacted], 2021)

Compound	Concentration	No. of	Genotoxicity	Cytotoxicity

	[µg/mL]	binucleated cells scored	% Binucleated cells containing micronuclei[a]	Mean CBPI	Mean cytostasis [%]
Without metabolic activation; 4-hours treatment					
Solvent					
Water	0	4000	0.25	1.85	0
HCD[#] mean ± SD			0.40 ± 0.16		
Range (95% control limits)			*0.08-0.72*		
Test item					
	277.5	2000	0.10	1.84	1
	555.0	2000	0.15	1.87	0[x]
	1110.0	2000	0.15	1.84	2
Positive control					
MMC	0.2	2000	**8.45**[***]	1.66	23
HCD[#] mean ± SD			3.71 ± 1.25		
Range (95% control limits)			*1.21-6.21*		
With metabolic activation; 4-hours treatment					
Solvent					
Water	0	4000	0.08	1.95	0
HCD[#] mean ± SD			0.39 ± 0.21		
Range (95% control limits)			*0-0.81*		
Test item					
	277.5	2000	0.05	1.98	0[x]
	555.0	2000	0.10	1.89	6
	1110.0	2000	0.20	1.92	4
Positive control					
CP	6.0	2000	**2.75**[***]	1.43	55
HCD[#] mean ± SD			2.18 ± 0.61		
Range (95% control limits)			*0.96-3.40*		
Without metabolic activation; 24-hours treatment					
Solvent					
Water	0	4000	0.13	2.04	0
HCD[#] mean ± SD			0.35 ± 0.17		
Range (95% control limits)			*0.01-0.69*		
Test item					
	277.5	2000	0.05	1.98	6
	555.0	2000	0.15	2.15	0[x]
	1110.0	2000	0.10	2.04	0[x]
Positive control					
DC	0.075	2000	**2.50**[***]	1.49	53
HCD[#] mean ± SD			4.31 ± 1.75		
Range (95% control limits)			*0.81-7.81*		

[#]: Treatment and sampling time point not specified
HCD: Historical control data (January 2019-April 2020; n = 40 for all the HCD)

Compound	Concentration [µg/mL]	No. of binucleated cells scored	Genotoxicity	Cytotoxicity	
			% Binucleated cells containing micronuclei[a]	Mean CBPI	Mean cytostasis [%]

MMC: Mitomycin C; DC: Demecolcine; CP: Cyclophosphamide
***: statistically significantly increased, χ^2 test, $p < 0.001$
x: Cytostasis was defined 0 when the relative CBPI value is equal to or higher than the solvent control
a: The percentage of micronucleated cells determined in a sample of 2000 binucleate cells (4000 for vehicle)

III. CONCLUSION

Under the conditions of the test, AMPA did not induce any statistically significant increases in the frequency of binucleate cells with micronuclei in either the absence or presence of a metabolising system. The test item was therefore considered to be non-clastogenic and non-aneugenic to human lymphocytes *in vitro*.

Assessment and conclusion by applicant:
Negative for induction of micronuclei in human lymphocytes *in vitro*, in the presence and absence of metabolic activation.

The study was conducted compliant with GLP and according to OECD guideline 487 (2016). There were no deviations. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. AMPA (batch: 107785, purity: 99.2%) was negative for the induction of micronuclei (induction of chromosome breaks and/or gain or loss) in human peripheral lymphocytes with and without metabolic activation under the conditions of this *in vitro* micronucleus assay.

B.6.8.1.1.8. Genotoxicity – *in vivo*

Study 1

1. Information on the study

Data point	CA 5.8.1/026
Report author	[redacted]
Report year	1993
Report title	Mutagenicity test: Micronucleus test with AMPA, batch 286-JRJ-73-4
Report No	13268
Document No	146-GLY
Guidelines followed in study	OECD 474 (1983), US EPA 40 CFR part 700 (F) § 798.5395 (1987)
Deviations from current test guideline (OECD 474, 2016)	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. However, in the present study only 1000 polychromatic erythrocytes were evaluated. The percentage of polychromatic erythrocytes among total erythrocytes was determined for 200 erythrocytes instead of 500 erythrocytes. Only a single dose was tested. Although bone marrow toxicity was observed, no lower dose levels were additionally tested. In addition, no 24-hour control animals were included (first sampling time point). No data on proficiency and/or historical control data were provided and acceptance and evaluation criteria were not specified 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 lack of historical control data.

2. Full summary

Executive summary

AMPA, metabolite of glyphosate , was tested for its clastogenic potential in NMRI mice in a micronucleus test. Based on the results of an initial toxicity study in which no signs of toxicity became evident, the micronucleus test was performed at a dose level of 5000 mg/kg bw in three groups of animals.

The test item was dissolved in 0.9 % NaCl with 1 % carboxymethyl cellulose and administered as a single dose to groups of 5 mice/sex at a constant dosage volume of 20 mL/kg bw. Similar constituted groups of 5 mice/sex received the positive (cyclophosphamide, 30 mg/kg bw) or vehicle control (0.9 % NaCl with 1 % carboxymethyl cellulose).

Bone marrow sampling of the test item treated groups was performed 24, 48 and 72 hours after treatment. Positive and vehicle control animals were sacrificed 24 and 48 hours after dosing, respectively. Smears were prepared from the femoral bone marrow of each animal and 1000 immature erythrocytes (PCE) per animal were scored for the presence of micronuclei. The percentage of PCEs in 200 erythrocytes was calculated. In addition, the number of micronucleated normochromatic erythrocytes (NCEs) was determined.

Oral administration of 5000 mg/kg bw AMPA was associated with a clear depression of erythropoiesis, as the ratio of PCE to NCE was significantly decreased when compared to solvent control animals.

Upon treatment with AMPA there was no statistically significant increase in the frequency of micronucleated PCEs at any sampling time point when compared to those of control animals. The incidence of micronucleated PCEs in the solvent and positive control groups were in accordance with the laboratory's historical control data, confirming the sensitivity of the test and demonstrating the capability of the test animals to respond to mutagenic substances.

Based on the experimental findings of this study, AMPA, metabolite of glyphosate is negative for cytogenetic effects in bone marrow in mice *in vivo*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA, metabolite of glyphosate
Description:	White powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark) was guaranteed for at least 3 years. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	1 % carboxymethyl cellulose (CMC) in 0.9 % NaCl

2. Control materials

Solvent (vehicle) control:	1 % carboxymethyl cellulose (CMC) in 0.9 % NaCl
Positive control:	Cyclophosphamide, 30 mg/kg bw

3. Test animals:

Species:	Mouse
Strain:	Bom:NMRI
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	8 weeks
Weight at dosing:	25 - 37 g
Acclimation period:	5 days

Diet/Food:	Altromin 1314 (Chr. Petersen Ltd., Ringsted, Denmark), *ad libitum*
Water:	Tap water acidified with hydrochloric acid to pH 2.5, *ad libitum*
Housing:	In groups of 5/sex in Macrolon type III cages measuring 420 x 260 x 150 mm with pinewood softwood sawdust bedding.

4. Environmental conditions:

Temperature:	21 ± 3 °C
Humidity:	55 ± 15 %
Air changes:	Approximately 10/hour
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	5000 mg/kg bw
Concentrations:	250 mg/mL
Dose volume:	20 mL/kg bw
Number of animals:	3/sex
Route of administration:	Oral gavage

b) Main micronucleus test

Dose levels:	5000 mg/kg bw
Concentrations:	250 mg/mL
Dose volume:	20 mL/kg bw
Number of animals:	5/sex/group (3 groups in total)
Route of administration:	Oral gavage

B. STUDY DESIGN AND METHODS

3. Dates of experimental work: 26 Oct - 12 Nov 1992
Finalisation date: 18 Feb 1993

4. Animal assignment and treatment:

Preliminary toxicity study:
AMPA, metabolite of glyphosate was expected to be of low toxicity. To determine the maximum tolerated dose, a single dose of 5000 mg/kg bw was administered by oral gavage to 3 mice/sex. Each one male and one female mouse were sacrificed 24, 48 and 72 hours after dosing and bone marrow smears were prepared. For each animal, the percentage of polychromatic erythrocytes among the total of erythrocytes was determined for 200 erythrocytes.
Based on the results of the preliminary toxicity study, 5000 mg/kg bw was selected as dose level for the main micronucleus test.

Main micronucleus test:
Three groups of 5 mice/sex/group received a single dose of 5000 mg/kg bw by oral gavage at a constant dosage volume of 20 mL/kg bw. Similar constituted groups of 5 mice/sex received the vehicle (0.9 % NaCl with 1 % carboxymethyl cellulose) or the positive control (30 mg/kg bw cyclophosphamide, administered via intraperitoneal injection).
At 24, 48 and 72 hours after treatment, each one group of animals was sacrificed by cervical dislocation and bone marrow smears were prepared. Positive control animals were sacrificed 24 hours after treatment, whereas the vehicle control group was sacrificed 48 hours after dosing.

5. Slide preparation:

Immediately after sacrifice, the right femoral bone was dissected, the proximal end of the femur was cut and marrow cells were flushed out with foetal calf serum. After whirl-mixing, the bone marrow cells were

centrifuged and smears were prepared. The specimens were fixed in methanol and stained with May-Grünwald/Giemsa.

6. Slide evaluation:

Slides were randomly coded and examined by microscopical analysis. About 1000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. The percentage of PCEs in 200 erythrocytes was calculated. In addition, the number of micronucleated normochromatic erythrocytes (NCEs) was determined during the counting of 1000 PCEs.

7. Statistics:

The number of micronucleated PCEs in the test group were compared to the number found in the vehicle group. Statistical analysis was performed using the one-way analysis of variance performed on the values transformed to normal scores according to Blom's method[3].

8. Acceptance criteria:

Acceptance criteria were not specified in the study report.

9. Evaluation criteria:

Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

E. ANALYTICAL DETERMINATIONS

Analytical determinations of the test substance in vehicle were not performed.

F. PRELIMINARY TOXICITY STUDY

Clinical signs of systemic toxicity were not described in the study report. Between 32 – 46 % of PCE were found in the animals, indicating that erythropoiesis was not or only slightly affected.
Based on the results of the preliminary toxicity study, 5000 mg/kg bw was selected as dose level for the main micronucleus test.

G. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:
No mortality occurred.

Clinical signs of toxicity:
Clinical signs of toxicity have not been described in the study report.

Evaluation of bone marrow slides:

The percentage of polychromatic erythrocytes (PCEs) in AMPA-treated animals was statistically significantly lower than in control animals, indicating a clear depression of erythropoiesis and demonstrating that bone marrow toxicity was evident.
In addition, there was no statistically significant increase in the frequency of micronucleated PCEs (mPCEs) of test item-treated animals at any sampling time point when compared to those of control animals. Although there was a slight trend towards an increase in mPCE in test item-treated males with later sampling time points, the values remained within those of control animals, were not significant and standard deviations were comparably low. The incidence of micronuclei in the solvent and positive control groups were in accordance with the laboratory's historical control data, confirming the sensitivity of the test and demonstrating the capability of the test animals to respond to mutagenic substances.

Table B.6.8.1.1.8.1-1: Summary of genotoxicity data for AMPA obtained in the micronucleus test in mice ([redacted], 1993)

Treatment	Dose	Sampling	Males	Females

[3] Blom, 1958: Statistical Estimates and Transformed Beta Variables, New York: John Wiley and Sons, Inc.

	(mg/kg bw)	time	mPCE ± SD[#] /1000 PCE	mean %[#] PCE ± SD / 200 erythrocytes	mPCE ± SD[#] /1000 PCE	mean %[#] PCE ± SD / 200 erythrocytes
CMC	20 mL/kg bw	48 h	0.80 ± 0.45	36.40 ± 2.61	0.20 ± 0.45	34.40 ± 2.97
Test item	5000	24 h	0.20 ± 0.45	28.20 ± 5.63	0.20 ± 0.45	25.20 ± 1.79
Test item	5000	48 h	0.60 ± 0.55	28.80 ± 4.92	0.20 ± 0.45	28.40 ± 3.21
Test item	5000	72 h	0.80 ± 0.45	26.60 ± 5.03	0.40 ± 0.55	24.20 ± 1.64
CPA	30	24 h	12.40 ± 2.07	35.00 ± 2.55	13.08 ± 3.03	33.40 ± 4.16

mPCE: micronucleated polychromatic erythrocytes
CMC: carboxymethyl cellulose, solvent control; CPA: cyclophosphamide, positive control
#: all values were re-calculated based on raw data of individual animals provided in the study report.

III. CONCLUSIONS

Based on the experimental findings, AMPA, metabolite of glyphosate did not induce micronuclei in the bone marrow of mice *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In the present study, AMPA was negative for clastogenic effects in the bone marrow of male and female NMRI mice *in vivo*.
The study was conducted under GLP and in accordance with OECD guideline 474 (1983). There were a number of deviations when compared to the current OECD guideline 474 (2016). Only 1000 polychromatic erythrocytes (PCE) were investigated per animal. However, the number of micronucleated PCE did not exceed those of control animals, standard deviations were comparably low and the values were statistically not significant when compared to control conditions. Thus, the test result was considered to be clearly negative. In addition, the percentage of polychromatic erythrocytes among total erythrocytes was determined for 200 erythrocytes instead of 500 erythrocytes and control animals were not included for the first sampling time point. These and further deviations were considered to be of minor degree and to not compromise the validity of the study. Therefore, the study is considered valid and acceptable.

Assessment and conclusion by RMS:
It is agreed with the applicants conclusion that, in this test, AMPA was negative for clastogenic effects in the bone marrow of male and female NMRI mice *in vivo*. Bone marrow exposure of the test compound was demonstrated.
In contrast to the applicant, the RMS considers this study as acceptable but with restrictions (reliable with restrictions) due to the lack of historical control data.

The study was accepted in the previous evaluation (RAR, 2015).

Study 2

1. Information on the study

Data point	CA 5.8.1/027
Report author	[redacted]
Report year	1993
Report title	Mouse Micronucleus Study of AMPA
Report No	[redacted]-13243
Document No	[redacted]-90170 / [redacted]-90-404
Guidelines followed in study	The study was performed similarly to OECD 474 (2016)
Deviations from current test guideline (OECD 474, 2016)	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. However, in the present study only 1000 polychromatic erythrocytes

	were evaluated. The study was however in line with the OECD test guideline which was valid at the time of conduct of the study. The animals were dosed via intraperitoneal injection, which is not representative for a human route of exposure and therefore not a recommended route for administration according to the OECD guideline. A justification for the route of administration was not given. However, the route of exposure does not invalidate the outcome of the study and therefore this is not considered to impact the reliability of the study. Although there was no concurrent proof for bone marrow exposure, signs of systemic toxicity were observed and it can be assumed that bone marrow exposure was achieved by the intraperitoneal injection. Historical control data for positive control animals were not included. HCD was available for the negative controls. Acceptance criteria were not specified and evaluation criteria mentioned were inconsistent with those recommended by the guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 2a) **Conclusion AGG:** Although the study showed some deviations to the current OECD test guideline it is in accordance with the test guideline that was valid at the time of conduct of the study and therefore considered acceptable.

2. Full summary

Executive summary

AMPA, metabolite of glyphosate, was tested for its clastogenic potential in CD-1 mice in a micronucleus test. In two initially performed toxicity studies, in which clinical signs of toxicity and mortality were observed at ≥ 606 mg/kg bw, a LD_{50} of 1357.7 mg/kg bw was determined. 1000 mg/kg bw (representing approx. 74 % of the LD_{50}) was selected as top dose level for the main micronucleus study.

The test item was dissolved in corn oil and administered as a single intraperitoneal injection to groups of 5 mice/sex at dose levels of 100, 500 and 1000 mg/kg bw. Similarly constituted groups of 5 mice/sex received the solvent or the positive control (cyclophosphamide, 40 mg/kg bw).

Bone marrow sampling of the test item and solvent control treated groups was performed 24, 48 and 72 hours after treatment. Positive control animals were sacrificed 24 hours after dosing. Smears were prepared from the femoral bone marrow of each animal and 1000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. In addition, the percentage of PCEs among a total of 1000 erythrocytes was determined.

Intraperitoneal injection of AMPA induced signs of systemic toxicity, evident as listlessness, in 6/15 males and 1/15 females at 500 mg/kg bw and in 13/15 males and 3/15 females at 1000 mg/kg bw. The symptoms were observed until study termination. In addition, body weight development was statistically significantly impaired in mid and high dose males of the 48-hour sampling time point and in mid dose group females of the 24-hour and 72-hour sampling time point. The findings were attributed to treatment and considered toxicologically relevant.

Although bone marrow exposure can be assumed, due to the observations on systemic toxicity, there was no bone marrow toxicity in terms of a decreased PCE/total erythrocyte ratio observed in any AMPA treated group and for any sampling time point.

A statistically significant increase in the frequency of micronucleated PCEs when compared to solvent controls was observed for females at 100 mg/kg bw at the 72 hour sampling time point. The values were within the range of the laboratories historical control data and showed no dose-response relationship, therefore the finding was not attributed to treatment. There was no statistically significant increase in the mean number of micronucleated PCEs observed for any other male or female dose group at any other sampling time point.

The incidence of micronucleated PCEs in the solvent controls remained within the range of historical control data, whereas those of the positive controls showed a marked increase of statistical significance. The results obtained for the solvent and positive controls thus confirmed the sensitivity of the test and demonstrated the capability of the test animals to respond to mutagenic substances.

Based on the experimental findings, AMPA, metabolite of glyphosate is negative for cytogenetic effects in bone marrow in mice *in vivo*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminomethylphosphonic acid (AMPA, metabolite of glyphosate)

Identification:	AMPA
Description:	White solid
Lot/Batch #:	HET-9001-1463T (Test sample T900031)
Purity:	94.38 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark) was guaranteed until the expiry date Jan 1993. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	Corn oil

2. Control materials

Solvent (vehicle) control:	Corn oil
Positive control:	Cyclophosphamide monohydrate, 40 mg/kg bw in Hank's balanced salt solution (HBSS)

3. Test animals:

Species:	Mouse
Strain:	CD-1
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	7 – 10 weeks
Weight at dosing:	27.9 – 38.7 (males) and 21.5 – 33.2 (females)
Acclimation period:	Minimum 7 days
Diet/Food:	Purina Certified Laboratory Rodent Chow® No. 5002 (Purina Mills Inc., St. Louis, Missouri), *ad libitum*
Water:	Tap water from the public water supply, *ad libitum*
Housing:	Individually in stainless steel cages with stainless steel mesh bottoms.

4. Environmental conditions:

Temperature:	64 - 79 °F (18 – 26 °C)
Humidity:	40 - 70 %
Air changes:	Not specified
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

c) First preliminary toxicity study

Dose levels:	1000 and 5000 mg/kg bw
Concentrations:	Not specified
Dose volume:	10 mL/kg bw
Number of animals:	2/sex/dose level
Route of administration:	Intraperitoneal injection

d) Second preliminary toxicity study

Dose levels:	303, 606, 1250, and 2500 mg/kg bw
Concentrations:	Not specified
Dose volume:	10 mL/kg bw
Number of animals:	3/sex/dose level
Route of administration:	Intraperitoneal injection

e) Main micronucleus test

Dose levels:	100, 500 and 1000 mg/kg bw
Not specified	Not specified
Dose volume:	10 mL/kg bw
Number of animals:	5/sex/group
Route of administration:	Intraperitoneal injection

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 20 Aug – 24 Oct 1990
Finalisation date: 08 Dec 1993

2. Animal assignment and treatment:

Preliminary toxicity studies:

An initial range-finding experiment was conducted using 2 mice/sex/dose level. A single dose of 1000 or 5000 mg/kg bw was administered via intraperitoneal injection at a constant dosage volume of 10 mL/kg bw. Afterwards, the animals were observed for mortality and clinical signs of toxicity during a 4-days observation period.

Based on the results of the first preliminary toxicity study, a second toxicity study was performed prior to the main micronucleus assay in order to estimate the maximum tolerated dose (MTD). In the second preliminary study, groups of 3 mice/sex were injected with test item concentrations in the range of 303 - 2500 mg/kg bw. After dosing, the animals were also observed for clinical signs of toxicity and mortality. Each 1/3 males and 1/3 females of the 303 and 606 mg/kg bw dose group and 1/3 females of the 1250 mg/kg bw dose group were sacrificed 24 hours after dosing for erythrocyte evaluation (data not provided in study report). The remaining animals were observed for three further days.

LD_{50} values and the maximum tolerated dose were estimated with the data obtained in both preliminary experiments. Based on the findings of the two initial toxicity studies, the dose levels for the main micronucleus study were selected.

Main micronucleus test:

Groups of 5 mice/sex received a single AMPA dose of 100, 500 or 1000 mg/kg bw via intraperitoneal injection. The test item was administered at a constant dosage volume of 10 mL/kg bw. Similarly constituted groups of 5 mice/sex received the vehicle (corn oil) or the positive control (40 mg/kg bw cyclophosphamide).

Each one group of test item-treated or vehicle control animals was sacrificed by cervical dislocation 24, 48 and 72 h after treatment, followed by preparation of bone marrow smears. Positive control animals were sacrificed 24 hours after treatment.

3. Slide preparation:

After sacrifice, the femora of the animals were removed, cut and marrow cells were flushed out with fetal bovine serum. The cells were centrifuged and smears were prepared. For each animal two slides were prepared. The slides were allowed to air-dry over night and stained with Wright-Giemsa Stain Pak by using a Hema-Tek II slide staining machine.

4. Slide evaluation:

Slides were randomly coded prior to analysis. For each animal, a total of 1000erythrocytes was evaluated for the amount of polychromatic (PCE) among total erythrocytes and 1000 PCEs were scored for the presence of micronuclei. PCEs containing more than one micronucleus were scored as single micronucleated PCE (mPCE). In a few cases significant discordance in mPCE frequency were initially observed between two slide scorers (e.g. a difference of 4 or more mPCE). In these cases slides were rescored to determine if the discordance was reproducible and the rescored values were used for reporting and analysis. Scoring data were used to calculate, for each animal, the ratio of PCEs to total erythrocytes (PCEs plus NCEs) per 1000 erythrocytes and the number of mPCEs per 1000 PCEs.

5. Statistics:

LD_{50} estimates were calculated using the Probit method on toxicity range-finding data. The individual test animal was used as the individual unit for analysis of micronucleated polychromatic erythrocyte (PCE) frequency, PCE/total erythrocyte ratio and body weight change. Micronucleated PCE frequencies per animal were transformed as the square root prior to analysis (Snedecor and Cochran[4] (1967) and MacGregor[5] et al. (1987)). PCE/total erythrocyte ratios were not transformed. A Dunnett's test (one sided) was used for comparison of treatment group and positive control values with solvent control values.

6. Acceptance criteria:

Acceptance criteria were not specified in the study report.

7. Evaluation criteria:

Evaluation criteria were not specified in the study report. A test item was considered to induce a statistically significant, treatment-related response in micronuclei formation if the following criteria were met:

- There was a dose- and time-dependent effect, which was consistent with a treatment-induced response.
- The degree of the response was in relation to both concurrent and historical negative and positive control data.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the present study.

B. PRELIMINARY TOXICITY STUDY

In the first preliminary toxicity study, mortality was observed in 2/2 males and 1/2 females at 1000 mg/kg bw 3 and 4 days following treatment and in 2/2 males and 2/2 females at 5000 mg/kg bw within the first two days after dosing. During the 4-day observation period until death, all animals showed ante-mortem signs of systemic toxicity which included listlessness and unresponsiveness.

In the second preliminary toxicity study, mortality was noted in 1/2 males and 0/2 females at 606 mg/kg bw on study Day 4, in 2/3 males and 0/2 females at 1250 mg/kg bw on study Days 2 and 3 and in 2/3 males and 1/3 females at 2500 mg/kg bw on study Day 2, respectively. Clinical signs of toxicity, evident as listlessness or unresponsiveness were noted in 2/2 males at 606 mg/kg bw on study Days 3 and 4, in 2/3 males and 3/3 females at 1250 mg/kg bw throughout the whole observation period and in 3/3 males and 2/3 females at 2500 mg/kg bw throughout the whole observation period.

The LD_{50} for male and female mice combined was calculated to be 1357.7 mg/kg bw. Based on the results of the two preliminary toxicity studies, 1000 mg/kg bw (representing approx. 74 % of the LD_{50} value) was selected as maximum tolerated dose level for the main micronucleus test.

The RMS notes that the observed mortalities for the treated males seem consistent over the two preliminary studies. In contrast, mortalities of the treated females seem inconsistent. In the first preliminary experiment, a death was observed for one female at 1000 mg/kg bw, whereas no deaths occurred in the second experiment at 1250 mg/kg bw. Results might suggest a possible sex difference in toxicity, however, considering that the difference in lowest dose levels with lethality between males and females (606 mg/kg bw and 1000 mg/kg bw, respectively) was not large, 1000 mg/kg bw was selected as highest dose level in both sexes for the main experiment.

[4] Snedecor, G.W. and Cochran, W. G. (1967) Statistical Methods, 6th edition, 223-226 and 325-327, Iowa State Press, Ames, Iowa.

[5] MacGregor, J.T., Heddle, J.A., Hite, M., Margolin, B. H., Ramel, C., Salaake, M.F., Tice, R.R. and Wild, D. (1987). Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. *Mutation Research 189* 103-112.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:
No mortality occurred.

Clinical signs of toxicity:
Listlessness was observed in 6/15 males and 1/15 females at 500 mg/kg bw and in 13/15 males and 3/15 females at 1000 mg/kg bw. The symptoms were present until study termination up to 72 hours after dosing. No clinical signs of toxicity were observed at the 100 mg/kg bw dose group or in solvent control animals.

Body weight development:
Statistically significant decreases in mean body weight change were observed for the mid (500 mg/kg bw) and high dose (1000 mg/kg bw) test group in males sacrificed 48 hours after dosing and for the mid dose group in females sacrificed 24 and 72 hours after dosing. The impaired body weight development in males of the 48-hour sampling time point exhibited a dose-response pattern. Thus, the observations were attributed to treatment and considered toxicologically relevant.

Table B.6.8.1.1.8.2-1: Body weight development after treatment with AMPA, observed in the micronucleus test in mice ([redacted], 1993)

Treatment	Dose (mg/kg bw)	Sampling time	Mean BW change [g] mean ± SD	
			Males	Females
Corn oil	10 mL/kg bw	24 h	-0.8 ± 0.5	-0.6 ± 0.5
		48 h	-0.8 ± 0.6	-0.9 ± 0.4
		72 h	-0.5 ± 1.4	-0.2 ± 0.8
Test item	100	24 h	-0.5 ± 1.3	-1.4 ± 1.0
		48 h	-1.5 ± 0.4	-0.8 ± 0.6
		72 h	-0.3 ± 1.6	-0.6 ± 0.8
	500	24 h	-1.9 ± 0.7	**-1.6 ± 0.5***
		48 h	**-2.2 ± 1.2***	-1.1 ± 1.2
		72 h	-2.1 ± 1.9	**-2.0 ± 0.8****
	1000	24 h	-1.4 ± 0.16	-1.4 ± 0.8
		48 h	**-3.2 ± 1.0****	-1.6 ± 1.2
		72 h	-1.0 ± 0.9	-0.4 ± 0.7
CPA	40	24 h	-1.2 ± 0.3	-0.5 ± 0.4

CPA: cyclophosphamide, positive control

BW: body weight

* $p \leq 0.05$ and ** $p \leq 0.01$ by one-sided Dunnett's test

Evaluation of bone marrow slides:
There was no statistically significant decrease in the mean polychromatic erythrocytes (PCE)/total erythrocytes ratio for any of the AMPA treated or control groups at any sampling time point, indicating that no bone marrow toxicity was evident. However, as clinical signs of toxicity were observed in AMPA-treated animals of the 500 and 1000 mg/kg bw groups, it can be assumed that bone marrow was exposed.
A statistically significant increase in the frequency of micronucleated PCEs when compared to solvent controls was noted for females at the low dose level (100 mg/kg bw) at the 72 hour sampling time point. The values were within the range of the laboratories historical control data and showed no dose-response relationship, therefore the finding was not considered to reflect a treatment-related effect. There was no statistically significant increase in the mean number of micronucleated PCEs observed for any other male or female dose group at any other sampling time point.
The incidence of micronuleated PCEs in the solvent controls remained within the range of historical control data. The positive control (cyclophosphamide) yielded expected positive responses in micronucleated PCE frequency, indicating the adequacy of the experimental conditions. It is noted that historical control data of the positive control are not included in the study report.

Table B.6.8.1.1.8.2-2: Summary of genotoxicity data for AMPA obtained in the micronucleus test in mice ([redacted], 1993)

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD /1000 PCE	PCE/total erythrocyte ratio mean ± SD	mPCE ± SD /1000 PCE	PCE/total erythrocyte ratio mean ± SD
Corn oil	10 mL/kg bw	24 h	0.2 ± 0.4	0.40 ± 0.05	1.0 ± 1.4	0.40 ± 0.10
		48 h	0.6 ± 1.3	0.48 ± 0.05	0.4 ± 0.9	0.50 ± 0.07
		72 h	0.2 ± 0.4	0.56 ± 0.02	0.0 ± 0.0	0.52 ± 0.04
	HCD mean[1] *range*	24 -72 h	0.954 ± 1.303 *0.00 - 2.40*		1.421 ± 2.676 *0.00 - 7.40*	
Test item	100	24 h	0.2 ± 0.4	0.42 ± 0.07	0.8 ± 0.8	0.48 ± 0.06
		48 h	0.0 ± 0.0	0.44 ± 0.07	0.2 ± 0.4	0.42 ± 0.08
		72 h	0.0 ± 0.0	0.51 ± 0.02	1.6 ± 1.1*	0.63 ± 0.09
	500	24 h	0.1 ± 0.3	0.43 ± 0.05	2.0 ± 2.9	0.38 ± 0.12
		48 h	0.6 ± 0.9	0.54 ± 0.09	0.2 ± 0.4	0.49 ± 0.02
		72 h	0.0 ± 0.0	0.50 ± 0.07	0.8 ± 0.8	0.62 ± 0.04
	1000	24 h	0.8 ± 1.3	0.42 ± 0.09	0.8 ± 0.8	0.41 ± 0.06
		48 h	0.2 ± 0.4	0.46 ± 0.07	0.0 ± 0.0	0.45 ± 0.08
		72 h	0.0 ± 0.0	0.51 ± 0.03	0.4 ± 0.9	0.58 ± 0.06
CPA	40	24 h	18.3 ± 10.9**	0.43 ± 0.06	12.0 ± 12.3*	0.48 ± 0.05

mPCE: micronucleated polychromatic erythrocytes

CPA: cyclophosphamide, positive control

HCD: historical control data on mean mPCE/1000 PCEs (24 - 72 h sampling combined data), generated in the testing laboratory (time frame not specified)

* $p \leq 0.05$ and ** $p \leq 0.01$ by one-sided Dunnett's test. Square root transformed data used for statistical analysis of mPCE

[1] Males: combined HCD based on 65 animals (24 h, 13 studies), 70 animals (48 h, 13 studies), and 45 animals (72 h, 9 studies). Females: combined HCD based on 50 animals (24 h, 10 studies), 50 animals (48 h, 10 studies), and 50 animals (72 h, 9 studies).

Note RMS: The applicant is kindly asked to provide more detailed information on the historical negative control data, for instance when data were generated.

III. CONCLUSIONS

Based on the experimental findings, AMPA, metabolite of glyphosate did not induce micronuclei in the bone marrow of mice *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was conducted in compliance with GLP and similar to OECD guideline 474 (2016), although some deviations became evident. The number of polychromatic erythrocytes (PCE) evaluated was less than the 2000 PCE which are recommended by the current guideline. Based on the evaluation of 1000 PCE/animal, the test item was considered negative for clastogenic effects in the bone marrow of male and female CD-1 mice *in vivo*. Although there was a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes (PCE) in females at the low dose level (100 mg/kg bw) at the 72 hour sampling time point, the values were within the range of the laboratories historical control data and showed no dose-response relationship. Thus, the finding was not considered to reflect a treatment-related effect. It can be assumed that the negative test result obtained in the study would be the same if more cells were evaluated.

With regard to the positive controls, male animals showed a distinct increase in mPCEs of statistical significance. In 2/5 positive control females no induction of micronuclei in PCEs was observed, while a marked increase in mPCEs was observed for the remaining 3/5 positive control females. This finding was not

mentioned or further discussed in the study report. However, a statistically significant increase in mPCE was still observed when combining the data of all 5 animals. Therefore, the results obtained were considered to confirm the sensitivity of the test and to demonstrate the capability of the test animals to respond to mutagenic substances.
Although bone marrow toxicity, indicated by a decreased PCE to normochromatic erythrocyte (NCE) ratio, was not observed, clinical signs of toxicity indicated systemic availability of the test substance. In addition, the test item was administered by intraperitoneal injection, therefore it can be assumed that bone marrow exposure was achieved.
Further deviations were of minor degree and considered to not compromise the validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:
It is agreed that under the test conditions AMPA was not genotoxic. This is in line with the previous assessment (RAR, 2015).

B.6.8.1.1.9. Developmental toxicity

Study 1

Data point	CA 5.8.1/028
Report author	[redacted]
Report year	1992
Report title	AMPA: Teratogenicity study in rats
Report No	7891
Document No	124-GLY
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision F, 83-3
Deviations from current test guideline (OECD 414, 2018)	Duration of treatment was shorter than required (GD6-16). The following endpoints were not assessed: weight and histopathological changes of the thyroid glands, foetal anogenital distance (AGD), indication of incomplete testicular descent/cryptorchidism in male foetuses; blood samples from dams to assess thyroid hormons (T4, T3 and TSH) were not collected.
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. Some deviations were noted from the current OECD test guideline. However, the study was in accordance with the OECD test guideline valid at the time of conduct of the study.

2. Full summary

Executive summary

This developmental study in rats was performed in two successive replicates, the first containing 10 mated females per treatment group, the second a further 15 females per group. For each replicate, mated female Sprague Dawley rats were randomised into four treatment groups. The animals were dosed by gavage once daily over Days 6-16 inclusive of gestation, where Day 0 was the day of detection of mating. Dose levels of test material were as follows: 0, 100, 350 and 1000 mg/kg bw/day. The animals were monitored during gestation for clinical signs of toxicity and for body weight and food consumption performance. They were sacrificed on Day 20 of gestation and the conceptuses were evaluated. The live foetuses were subsequently examined for developmental abnormalities and variants of the viscera and skeleton, including the state of skeletal ossification.
There was no evidence of toxicity in either the dam or embryo-fetal development at any of the dose levels applied.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	AMPA
Description:	white powder
Lot/Batch number:	286-JRJ-73-4
Purity:	99.2 %
CAS#:	1066-51-9
Stability of test compound:	Stable until 09-Jun-1995
2. Vehicle:	0.5 % carboxymethylcellulose (CMC) in distilled water
3. Test animals:	
Species	Rat
Strain	Sprague-Dawley CD
Age/weight on arrival	Approximately 9 weeks / approx. 250 g
Source	[redacted]
Housing	Individually after mating, in polypropylene cages (42 x 27 x 20 cm), with a stainless steel grid bottom, top and food hopper
Acclimatisation period	3-5 days

Diet	Rat and Mouse Breeder Diet No. 3 (Expanded) SQC (Special Diets Services (SOS) Limited), *ad libitum*
Water	Mains water, *ad libitum*
Environmental conditions	Temperature: 20 ± 2°C Humidity: 50 ± 15 % Air changes: 15 - 20 changes / hour Photoperiod: 12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

Dates of Experimental work: between 27 February 1992 and 3 September 1992

In-life dates: Not reported (QA audits conducted between March 1992 and July 1992)

Mating procedure: Mating was on the basis of two females to each male, female siblings not being paired with the same male. For each female, cohabitation with a male was continuous until mating was detected. A vaginal lavage was examined each morning and the day of detection of sperm in the lavage, or of a copulatory plug *in situ*, was considered as Day 0 of gestation. A record was kept of the male which inseminated each female.

Animal assignment: On detection of mating, the females were re-housed in an individual cage and allocated to a treatment group using a computer-generated series of randomly sequenced numbers representing the treatment groups.

Dose selection rationale: Dose levels were agreed with the Sponsor after evaluation of existing toxicity data. These data indicated that the material in rats has a low toxic potential, with a lowest effect level (LEL) of 1200 mg AMPA/kg bw/day by dietary administration and an oral LD_{50} of approximately 8000 mg AMPA/kg bw. It was considered appropriate to proceed with the teratogenicity study using the conventional 'limit' dose of 1000 mg AMPA/kg bw/day as the highest dose level.

Dose preparation and analysis: The test item was formulated as a part-solution, part-suspension, the vehicle being a 0.5 % solution of carboxymethylcellulose (CMC) in distilled water. Fresh formulations were prepared daily, each concentration being prepared separately. The requisite quantity of test item was weighed into a labelled container. The necessary volume of vehicle was added, and mixing was by means of a Silverson laboratory mixer/emulsifier. The undissolved material was maintained in suspension during dosing procedures by means of a magnetic stirrer.

Samples of dosing formulations were analysed at the [redacted] under the provisions of [redacted] Project No. 353917 (Method Establishment, homogeneity, accuracy and stability) and under Project No. 490421 (concentration accuracy and homogeneity of actual dosing formulations).

For each study replicate, triplicate samples of 1 mL were taken from each concentration on the first day of treatment, then again near the end of the treatment period.

Dosage administration: The mated females were dosed once daily, at approximately the same time each day, over Days 6-16 inclusive of gestation, where Day 0 was the day of detection of mating.
Doses were administered orally, by gavage, at a volume of 10 mL/kg bw, using a steel dosing cannula. The volume of formulation administered to each female was determined each day by the weight of that animal as measured at the time of administration.

Clinical observations: All animals were checked for viability at the beginning of each day and again as late as possible on each day. All animals were examined for reaction to treatment on each day. The nature, onset, duration and intensity of any signs were recorded, based on a routine examination conducted 1-1.5 h after dosing, with any necessary follow-up examinations.

Bodyweight: Individual body weights were recorded on Days 0, 6, 9, 13, 17 and 20 of gestation.

Food consumption: The weight of the food consumed by each mated female was recorded daily throughout, commencing on Day 4 of gestation (weighed quantity first offered on Day 3 of gestation).

Terminal investigations: On Day 20 of gestation, the animals were killed by carbon dioxide asphyxiation. The contents of the thoracic and abdominal cavities were examined macroscopically for abnormalities. Any abnormal tissue was sampled and preserved in neutral buffered 10 % formalin, to facilitate any necessary histological analysis. The reproductive tract was removed and weighed intact, then opened and the contents were examined. The number of corpora lutea in each ovary and the number and position of all implantation sites in the uterus were recorded. Each implant was classified as being live, a foetal death (death judged to have occurred after ca. Day 16 of gestation), a late embryonic death (death judged to have occurred in the period ca. Day 12- Day 16) or an early embryonic death (death judged to have occurred prior to ca. Day 12).

Foetal observations: Each live foetus was individually identified within the litter and its weight was recorded. The foetuses were examined for externally visible abnormalities prior to fixation. Approximately one half of the foetuses from each uterus were fixed in methylated ethyl alcohol, and the remaining half in Bouin's fluid. Those foetuses fixed in alcohol were subsequently examined by open dissection for visceral abnormalities. The eviscerated carcasses were then cleared in potassium hydroxide and glycerol, and the skeletons were stained with Alizarin Red S. Skeletal structures in these foetuses were examined for abnormalities and variants, including state of ossification.

Those foetuses fixed in Bouin's fluid were examined for soft tissue abnormalities and variants by means of a freehand razor blade sectioning technique derived from that of Wilson.

The sex of each foetus was determined during the dissection procedures.

Statistical analyses: It was considered unnecessary to conduct any formal statistical tests on the data from this study. Interpretation was able to be based on examination of the individual and group mean values.

II. RESULTS AND DISCUSSION

A: ANALYSIS OF DOSE FORMULATIONS

Dose formulation analysis data show that the formulations used in the study were prepared to an acceptable level of accuracy, were homogeneous, and were stable for at least 24 hours.

B: CLINICAL OBSERVATIONS

One female at 1000 mg/kg bw/day was difficult to dose on Day 13 of gestation; salivation was noted at dosing but had ceased by 1 h post-dosing. For a second high dose animal a hard mass was noted associated with the left scapula. Post-mortem examination identified the mass as a 5 x 4 mm cartilaginous/bony outgrowth from left scapula, apparently non-neoplastic. These findings were considered incidental and not associated with treatment. In addition to these, there were sporadic findings common to this strain of animals (e.g. patches of alopecia, skin scabbing), there having been no relationship to treatment in their incidence.

C. BODY WEIGHT

There was no effect of treatment with AMPA on maternal body weights.

Table B.6.8.1.1.9.1-25: AMPA: Teratogenicity study in rats ([redacted], 1992): Summary of maternal body weight and body weight gain.

	Dose level of AMPA (mg/kg bw/day)			
Body weight (g ± S.D.) Day post coitum	**0 (control)**	**100**	**350**	**1000**
6	303 ± 25	303 ± 19 (100%)	300 ± 27 (99%)	306 ± 23 (101%)
13	347 ± 27	347 ± 24 (100%)	344 ± 27 (99%)	348 ± 26 (100%)
17	387 ± 31	387 ± 28 (100%)	384 ± 30 (99%)	391 ± 31 (101%)
20	439 ± 37	438 ± 34 (100%)	439 ± 35 (100%)	447 ± 33 (102%)
Body weight gain (g ± S.D.) Day 6-17 post coitum	83.6 ± 11.3	83.5 ± 12.2 (100%)	83.8 ± 8.5 (100%)	85.0 ± 15.3 (102%)

S.D. = Standard deviation

D. FOOD CONSUMPTION

There was no effect of treatment with AMPA on maternal food consumption.

Table B.6.8.1.1.9.1-2: AMPA: Teratogenicity study in rats ([redacted], 1992): Summary of maternal food consumption (g/animal/day)

Food consumption Day post coitum	Dose level of AMPA (mg/kg bw/day) 0 (control)	100	350	1000
6	31	30 (97%)	30 (97%)	29 (94%)
13	34	33 (97%)	34 (100%)	35 (103%)
17	38	36 (95%)	38 (100%)	39 (103%)
19	30	32 (107%)	34 (113%)	32 (107%)
Total consumption Days 6-17 post coitum	413	406 (98%)	415 (100%)	424 (103%)

E. NECROPSY

Gross pathology: Not reported.

Maternal performance:
There was no effect of treatment on any of the examined parameters of pregnancy performance. In particular, there were no notable inter-group differences in the incidence of intra-uterine death, or in mean foetal weight.

Table B.6.8.1.1.9.1-3: AMPA: Teratogenicity study in rats ([redacted], 1992): Intergroup comparison of maternal performance

Observation	Dose level of AMPA (mg/kg bw/day) 0 (control)	100	350	1000
No. of animals mated	25	25	25	25
No. of animals pregnant	25	25	24	24
No. of premature decedents	0	0	0	0
Pregnancy frequency (%)	100	100	96	96
Mean No. of corpora lutea ± SD	18.2 ± 2.0	17.5 ± 1.9	17.3 ± 1.7	17.9 ± 1.7
Mean No. of implantations ± SD	17.8 ± 1.7	17.0 ± 2.0	16.9 ± 1.9	17.5 ± 1.8
Pre-implantation loss (%)	2	3	3	2
Mean live implantations (%) ± SD	16.6 ± 2.0	15.3 ± 3.5	15.5 ± 2.8	16.8 ± 1.9
Mean dead implantations (%)± SD	1.2 ± 1.4	1.7 ± 2.8	1.4 ± 1.4	0.8 ± 0.8
Mean early embryonic deaths ± SD	1.1 ± 1.4	1.6 ± 2.6	1.1 ± 1.3	0.6 ± 0.8
Mean late embryonic deaths ± SD	0.2 ± 0.4	0.1 ± 0.4	0.3 ± 0.7	0.1 ± 0.3
Mean foetal deaths	0	0	0	0.04 ± 0.2
Live foetal sex ration (m:f)	1:0.92	1:1.01	1:1.11	1:0.92
Mean total uterus weight (g) ± SD	98.5 ± 11.5	89.7 ± 19.2	93.0 ±14.5	99.0 ± 11.1
Mean litter mean foetal weight (g) ± SD	3.76 ± 0.19	3.73 ± 0.21	3.78 ± 0.26	3.68 ± 0.18

SD = Standard deviation

F. FOETUSES

There was-no effect of AMPA on the number, growth or survival of the foetuses *in utero*.

Major and minor abnormalities of various kinds occurred sporadically in all treatment groups. None of the incidences exceeded the levels expected from this strain of animals in this laboratory, and no effect of treatment was noted.

Equally, the various parameters of skeletal ossification state were considered not to indicate an effect of treatment. None of the inter-group differences, taking parameters individually or collectively, were considered of sufficient magnitude to be of biological significance.

Table B.6.8.1.1.9.1-4: AMPA: Teratogenicity study in rats ([redacted], 1992): Group incidence of major foetal abnormalities

Observation	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	100	350	1000
	Incidence of foetuses (litters)			
Total no. of foetuses examined	414	383	372	402
Total no. of litters examined	25	25	24	24
Cleft palate, oedema; limb, digit and lung abnormalities ± diaphragmatic hernia	0	6 (1)	0	0
Right-sided aortic arch	0	0	0	1 (1)
Abnormalities of atlas and occipital bones	0	1 (1)	0	0
Interventricular septal defect	0	0	1 (1)	0
Partial situs inversus, interventricular septal defect	0	0	0	1 (1)
Forelimbs extended downwards ± carpal flexure, shortened and thickended ribs, shortened body oedema	5 (1)	6 (2)	0	0
Oedema, fused digits, misshapen genital papilla	0	0	0	1 (1)
Hydronephrosis	2 (2)	0	1 (1)	1 (1)

III. CONCLUSIONS

Treatment with AMPA at dose levels of up to 1000 mg/kg bw/day produced no evidence of toxicity in either the dam or the outcome of pregnancy.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study is considered acceptable although some endpoints that are requested in the OECD 414 TG from 2018 are not included in this study. Treatment with AMPA at dose levels of up to 1000 mg/kg bw/day produced no evidence of toxicity in either the dam or embryo-fetal development. The dose level of 1000 mg AMPA/kg bw/day was considered the no observed effect level (NOAEL) in this study for both maternal and developmental effects.

Assessment and conclusion by RMS:

Agreed with the conclusion by the applicant. No signs of prenatal developmental or maternal toxicity were reported up to 1000 mg/kg bw/day. Although there are minor limitations to the study, it is considered acceptable as the study was conducted in accordance with the OECD test guideline valid at the time of conduct of the study. This conclusion is in line with the RAR (2015).

Study 2

Data point	CA 5.8.1/029
Report author	[redacted]
Report year	1991
Report title	A dose range-finding developmental toxicity study of AMPA in rats
Report No	[redacted]-50146
Document No	n.a.
Guidelines followed in study	Not applicable for this dose range-finding study
Deviations from current test guideline	Not applicable for this dose range-finding study
Previous evaluation	Accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Supportive, Category 3a **Conclusion AGG:** The study is considered to be acceptable but with restrictions (reliable with restrictions) and acceptable as preliminary study only.

2. Full summary

The potential maternal toxicity and developmental toxicity of AMPA were evaluated in rats. AMPA in corn oil was administered to five groups of eight mated Sprague Dawley Crl:CD•BR rats once daily from gestation Days 6 through 15. Dosage levels were 125, 250, 500, 750 and 1000 mg/kg bw/day administered in a constant volume of 10 mL/kg bw. A concurrent control group, composed of eight mated females, received the vehicle, corn oil, on a comparable regimen at 10 mL/kg bw. The route of administration was oral by gastric intubation. Clinical observations and body weights were recorded. All animals were sacrificed on gestation Day 20 for a scheduled Caesarean section. The uteri and ovaries were examined and the numbers of foetuses, early and late resorptions, total implantations and corpora lutea were recorded. Mean gravid uterine weights and net body weight changes were calculated for each group. The kidneys, spleen and liver of each female were weighed. The foetuses were weighed, sexed, examined for external malformations and developmental variations and discarded.
All animals in the study survived to the scheduled necropsy. No compound related clinical findings were observed at any dose level. There were no adverse effects at any dose level on mean body weights, body weight gains, gravid uterine weights, net body weight changes and organ weights. Treatment with AMPA had no apparent adverse effect on intrauterine growth and survival, and there were no external malformations or developmental variations observed in this study.
Based on the results of this study, dose levels of 150, 400 and 1000 mg/kg bw/day were considered suitable for the subsequent definitive developmental toxicity study of AMPA in rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	AMPA
Description:	white solid
Lot/Batch number:	HET-9001-1463T
Purity:	94.38 %
Stability of test compound:	Stable until Jan 1993
2. Vehicle:	Corn oil
3. Test animals:	
Species	Rat
Strain	Sprague-Dawley CD
Age on arrival	Approximately 10 weeks
Body weight at initiation of dosing	265 - 278 g (group mean values)

Source	[redacted]
Housing	Individually, in wire-mesh cages suspended above card board (except for mating when animals were housed pairwise)
Acclimatisation period	4 weeks
Diet	Purina® Certified Rodent Chow #5002, *ad libitum*
Water	Drinking water, *ad libitum*
Environmental conditions	Temperature: 70 - 73 °F (corresponding to 21 - 23 °C) Humidity: 59 ± 86 % Air changes: approximately 10 changes / hour Photoperiod: 12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

In-life dates: 19 Jun 1990 – 14 Jul 1990

Mating procedure: At the end of the acclimatization period, animals judged to be in good health and meeting acceptable body weight requirements (a minimum of 220 grams) were placed in a suspended wire-mesh cage with a resident male from the same strain and source for breeding. Resident males were untreated, sexually mature rats utilized exclusively for breeding. A breeding record containing the male and female identification numbers and the dates of cohabitation was prepared. The females were approximately 14 weeks old when paired for breeding.
Positive evidence of mating was confirmed by the presence of a copulatory plug in the vagina or the presence of sperm in a vaginal smear. Each mating pair was examined daily. The day on which evidence of mating was identified was termed day 0 of gestation and the animals were separated.

Animal assignment: The mated females were consecutively assigned in a block design to groups containing eight rats each by the following randomization procedure. The first mated female and the appropriate gestation Day 0 designation were entered on the form and the female was assigned to group 1, the second mated female was assigned to group 2, and the third to group 3, etc. This process was continued daily until eight females were placed into each group. Body weight values ranged from 217 g to 285 g on Day 0 of gestation.

Dose preparation and analysis: An appropriate amount of the test item (AMPA) was weighed for each group into tared weigh boats and transferred to a mortar. The test material was triturated with corn oil, and ground with a pestle until a slurry was obtained. The slurry was transferred to a graduated cylinder via a series of vehicle rinses. A sufficient amount of vehicle was added to attain a volume of 250 mL. The cylinder was inverted several times to ensure adequate mixing and the contents were transferred to a properly labelled storage container. The cylinder was rinsed with an additional 100 mL of vehicle which was also added to the storage container. The preparations were then mixed for approximately 5 minutes on a Polytron PT6000 homogenizer to reduce particle size and to ensure proper mixing. The dosing preparations were visually inspected for homogeneity prior to dispensation for the first day of dose administration.
Preparations for all dose groups were made once at study start, and were stored at room temperature. The preparations were stirred using a magnetic stir plate and bar each day prior to dispensing and during the dosing procedure.
Analysis of dose formulation was not performed in this dose range-finding study.

Dose administration: The dose formulations were administered orally by gavage, via a 16-gauge stainless steel gavage cannula, once daily for 10 consecutive days initiating on gestation Day 6 and continuing up to and including Day 15 of gestation. A dosage volume of 10 mL/kg bw was used for all dose levels. The control animals received corn oil on a comparable regimen of 10 mL/kg bw. Individual dosages were based on the most recent body weights.

Clinical observations: All animals were observed twice daily for moribundity and mortality. Detailed clinical observations were recorded individually from Days 0 through 20 of gestation (prior to compound administration during the dosing period). Animals were observed for signs of toxicity approximately one hour after treatment throughout the dosing period; all significant findings were recorded.

Bodyweight and gravid uterine weight: Maternal body weights were recorded individually on gestation Days 0, 6, 9, 12, 16, 18 and 20. A group mean body weight was calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and for days 6-16, 16-20 and 0-20.

Gravid uterine weight, net body weight (the Day 20 body weight minus the weight of the uterus and contents) and net body weight change (the Day 0-20 body weight change minus the weight of the uterus and contents) were presented for each gravid female at the scheduled Caesarean section.

Food consumption: Food consumption was not recorded in this study.

Terminal investigations: All maternal animals were sacrificed by carbon dioxide inhalation on gestation Day 20. The thoracic, abdominal and pelvic cavities were opened by a ventral midline incision and the contents examined. In all instances, the post mortem findings were correlated with the ante mortem comments and any abnormalities were recorded. The uterus and ovaries were excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened and the number and location of all foetuses, early and late resorptions and the total number of implantation sites were recorded. Uteri with no macroscopic evidence of nidation were opened and subsequently placed in 10 % ammonium sulphide solution for detection of early implantation loss as described by Sa1ewski.
The liver, kidneys and spleen from each dam were excised, trimmed and weighed and all findings recorded. The carcasses of the dams, including all organs, were then discarded.

Foetal observations: A detailed external examination of each foetus was conducted to include, but was not limited to, the eyes, palate, and external orifices. Findings were recorded as either developmental variations or malformations. The weight and sex of each foetus were recorded and the foetuses were discarded.

Statistical analyses: All analyses were conducted using two-tailed tests for a minimum significance level of 5 % comparing each treated group to the vehicle control group. Each mean was presented with the standard deviation (S.D.) and the number of animals (N) used to calculate the mean. The following statistical tests were performed: Chi-square test with Yates' correction factor (foetal sex ratios); Fisher's Exact test (malformations and variations); Mann-Whitney U test (early and late resorptions, dead foetuses, post-implantation losses); One-way ANOVA with Dunnett's test (corpora lutea, total implantations, viable foetuses, foetal body weights, maternal body weights and weight changes, maternal net body weight changes and gravid uterine weights, selected organ weights).

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Dose formulation analysis was not performed in this dose range-finding study

B. CLINICAL OBSERVATIONS

All animals survived to the scheduled sacrifice on gestation Day 20. There were no apparent test item-related clinical effects observed in any of the AMPA treated groups. Faeces, which appeared grey in colour, were observed infrequently during the daily examinations at dose levels of 250 mg/kg bw/day and above. The predominant findings at the 1-hour post-dosing period included red staining around the nose, noted in one or two animals in each of the treated groups, and a low incidence of salivation at dose levels of 250 mg/kg bw/day and above. However, no dose-relationship was apparent and the limited incidence of these findings was not considered to be a conclusive indication of a treatment effect.
Other clinical findings (hair loss, soft stool, yellow anogenital, urogenital and proximal tail staining, rales, red staining or material around the vaginal area, nose and mouth, and decreased defaecation and urination) occurred similarly in the control and treated groups or were generally single occurrences.

C. BODY WEIGHT

No adverse effects on mean body weights or mean body weight gains were apparent in any dose group throughout the study. Mean gravid uterine weights and net body weight changes in the treated groups were also apparently unaffected by test item administration. No statistically significant differences were observed between the control group values and those of the AMPA-treated groups.

Table B.6.8.1.1.9.2-26: AMPA: DRF teratogenicity study in rats ([redacted], 1991a): Summary of maternal body weight, body weight gain and gravid uterine weight.

	Dose level of AMPA (mg/kg bw/day)					
	0 (control)	125	250	500	750	1000

No. of females	8	8	8	8	8	7
Body weights (g ± SD) Day post coitum						
0	240 ± 16.1 (100%)	246 ± 11.9 (103%)	253 ± 13.3 (105%)	240 ± 9.5 (100%)	243 ± 20.8 (101%)	243 ± 11.6 (101%)
6	266 ± 16.7 (100%)	269 ± 13.1 (101%)	278 ± 14.1 (105%)	265 ± 13.0 (100%)	270 ± 20.6 (102%)	269 ± 14.4 (101%)
12	277 ± 17.1 (100%)	287 ± 13.9 (104%)	293 ± 13.8 (106%)	279 ± 13.1 (101%)	281 ± 17.8 (101%)	282 ± 13.1 (102%)
16	302 ± 23.5 (100%)	314 ± 17.8 (104%)	316 ± 16.6 (105%)	300 ± 13.7 (99%)	309 ± 23.8 (102%)	308 ± 16.5 (102%)
20	365 ± 27.1 (100%)	377 ± 21.2 (103%)	379 ± 18.9 (104%)	359 ± 32.1 (98%)	374 ± 26.3 (102%)	369 ± 21.5 (101%)
Body weight gain (g ± SD) Day 6-16 post coitum	36 ± 8.9 (100%)	46 ± 11.1 (128%)	38 ± 8.7 (106%)	35 ± 11.1 (97%)	39 ± 7.2 (108%)	39 ± 4.7 (108%)
Gravid uterine weight (g ± SD)	73.9 ± 12.2 (100%)	78.9 ± 17.1 (107%)	77.1 ± 8.7 (104%)	69.8 ± 29.1 (94%)	77.2 ± 8.2 (104%)	78.0 ± 3.7 (106%)

SD = Standard deviation

D. FOOD CONSUMPTION

Food consumption was not recorded in this study.

E. NECROPSY

Gross pathology: At scheduled sacrifice on gestation Day 20, macroscopic necropsy findings were observed as follows. One female at 500 mg/kg bw/day had a pitted kidney while one control group female had a dilated renal pelvis containing white precipitate. The only other remarkable internal finding was an accessory spleen in one female at 250 mg/kg bw/day. These findings were considered not due to treatment.

Organ weights: Mean liver, kidney and spleen weights in the treated groups were similar to the control group, indicating no apparent adverse effect from AMPA administration.

Caesarean section data: The mean numbers and percentages of post-implantation losses and viable foetuses in the 125, 250, 500, 750 and 1000 mg/kg bw/day groups were similar to the control group. Mean foetal body weights in the treated groups were also comparable to the control group. No remarkable differences were observed between the control and treated groups in foetal sex ratios or the mean numbers of corpora lutea and implantation sites. It was noted that a single female at 1000 mg/kg bw/day was non-gravid; this was considered not due to treatment.

Table B.6.8.1.1.9.2-27: AMPA: DRF teratogenicity study in rats ([redacted], 1991a): Intergroup comparison of maternal performance and Caesarean section data

Observation	**Dose level of AMPA (mg/kg bw/day)**					
	0 (control)	**125**	**250**	**500**	**750**	**1000**
No. of animals mated	8	8	8	8	8	8
No. of premature decedents	0	0	0	0	0	0
No. of animals pregnant	8	8	8	8	8	7
Pregnancy frequency (%)	100	100	100	100	100	87.5
Mean No. of corpora lutea ± SD	16.8 ± 3.11	16.1 ± 2.23	17.3 ± 3.06	16.3 ± 3.33	16.9 ± 2.64	16.3 ± 2.29
Mean No. of implantations ± SD	14.9 ± 2.03	14.6 ± 3.02	15.5 ± 1.93	14.3 ± 5.12	15.1 ± 1.55	15.0 ± 1.29
Mean post-implantation loss ± SD (%)	0.9 ± 0.83 (6.1%)	0.4 ± 0.52 (3.2%)	1.1 ± 0.99 (7.1%)	0.9 ± 0.83 (16.4%)	0.9 ± 0.99 (5.0%)	0.4 ± 0.79 (2.6%)

Mean viable foetuses ± SD (%)	14.0 ± 2.27 (93.9%)	14.3 ± 3.28 (96.8%)	14.4 ± 1.69 (92.9%)	15.3 ± 1.50 (83.6%)	14.3 ± 1.83 (94.2%)	14.6 ± 0.98 (97.4%)
Mean dead foetuses ± SD (%)	0.0 ± 0.00 (0%)	0.0 ± 0.00 (0%)	0.0 ± 0.00 (0%)	0.0 ± 0.00 (0%)	0.0 ± 0.00 (0%)	0.0 ± 0.00 (0%)
Mean foetal weight (g) ± SD	3.5 ± 0.15 (100%)	3.7 ± 0.21 (106%)	3.5 ± 0.21 (100%)	3.4 ± 0.24 (97%)	3.5 ± 0.18 (100%)	3.6 ± 0.25 (103%)
Total No. of male:female foetuses	51:61	57:57	57:58	60:47	59:55	52:50

SD = Standard deviation

F. FOETUSES

There was-no effect of AMPA on the number, growth or survival of the foetuses *in utero*.

The number of foetuses (litters) available for evaluation numbered 112(8), 114(8), 115(8), 107(7), 114(8) and 102(7) in the control, 125, 250, 500, 750 and 1000 mg/kg bw/day groups, respectively. No external malformations or developmental variations were noted in this study.

III. CONCLUSIONS

The dose level of 1000 mg AMPA/kg bw/day was the no observed effect level in this study for both maternal and developmental effects. Based on the results of this study, dose levels of 150, 400 and 1000 mg/kg bw/day were considered suitable for the subsequent definitive developmental toxicity study of AMPA in rats.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This dose range-finding study is considered acceptable. Treatment with AMPA at dose levels of up to 1000 mg/kg bw/day produced no evidence of toxicity in either the dam or on any embryo-fetal development endpoint.

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. No toxicity was reported up to 1000 mg/kg bw/day.

Study 3

Data point	CA 5.8.1/030
Report author	[redacted]
Report year	1991
Report title	A developmental toxicity study of AMPA in rats
Report No	[redacted]-50159
Document No	M-645464-01-1
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision F, 83-3, JMAFF 59 NohSan No. 4200, OECD 414 (1981)
Deviations from current test guideline (OECD 414, 2018)	Duration of treatment was shorter than required (GD6-15). The following endpoints were not assessed: weight and histopathological changes of the maternal thyroid glands, foetal anogenital distance (AGD), indication of incomplete testicular descent/cryptorchidism in male foetuses; assessment of dams' thyroid hormones (T4, T3 and TSH)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered to be acceptable. Although some deviations were noted compared to the current test guideline the study was conducted in accordance with the OECD test guideline valid at the time of conduct of the study.

2. Full summary
Executive summary

The potential maternal toxicity and developmental toxicity of AMPA were evaluated in this study. AMPA in corn oil was administered orally by gavage to three groups of 25 mated Charles River Crl: CD BR female rats once daily from gestation days 6 through 15. Dose levels were 150, 400 and 1000 mg/kg bw/day administered in a constant volume of 10 mL/kg bw. A concurrent control group, composed of 25 mated females, received the vehicle on a comparable regimen at 10 mL/kg bw. Throughout gestation all females were observed twice daily for appearance and behaviour. Body weights and food consumption were recorded at appropriate intervals. On day 20 of gestation, all females were sacrificed for a scheduled Caesarean section. The liver, kidneys and spleen of each female were weighed. The uteri and ovaries were examined and the numbers of foetuses, early and late resorption, total implantations and corpora lutea were recorded. Mean gravid uterine weights and net body weight changes were calculated for each group. Foetuses were weighed, sexed and examined for external, skeletal and soft tissue malformations and developmental variations.

Maternal survival was unaffected by treatment at all dose levels. No treatment-related gross necropsy findings were observed. Clinical findings which appeared related to test item administration occurred at 400 and 1000 mg/kg bw/day and included mucoid faeces, hair loss and soft stool. Mean body weight gains at 1000 mg/kg bw/day were similar to the control group during the initial six days of dosing (gestation Days 6-9 and 9-12); food consumption in this group was slightly decreased compared to the control group during the initial three days of dosing and was slightly increased during gestation Days 9-12. During gestation Days 12-16, mean body weight gain at 1000 mg/kg bw/day was slightly reduced and food consumption was similar to the control group during this interval. Body weight gain and food consumption values at 150 and 400 mg/kg bw/day were comparable to the respective control values. Mean gravid uterine weights, net body weights and net body weight gains, and mean liver, kidney and spleen weights in the AMPA treated groups were similar to the values in the control group. The mean foetal body weight at 1000 mg/kg bw/day group was slightly decreased. No other indication of a developmental effect was apparent at any dose level. No foetal malformations or developmental variations which could be related to treatment were observed at any dose level.

In conclusion, a marginal degree of maternal toxicity and a marginal degree of developmental toxicity were observed at 1000 mg/kg bw/day; maternal body weight gain and food consumption values were slightly less than those in the control group for a short duration and mean foetal body weight was slightly decreased. Clinical findings (mucoid faeces, soft stool and hair loss) were the only maternal response at 400 mg/kg/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	AMPA
Description:	white solid
Lot/Batch number:	HET-9001-1463T
Purity:	94.38 %
Stability of test compound:	Stable until Jan 1993
2. Vehicle:	Corn oil
3. Test animals:	
Species	Rat
Strain	Sprague-Dawley CD
Age on arrival	71 days
Body weight at initiation of dosing	265 - 278 g (group mean values)
Source	[redacted]
Housing	Individually, in wire-mesh cages suspended above card board (except for mating when animals were housed pairwise)
Acclimatisation period	11 days
Diet	Purina® Certified Rodent Chow #5002, *ad libitum*
Water	Tap water, *ad libitum*
Environmental conditions	Temperature: 69 – 72°F (corresponding to 21 – 22 C) Humidity: 61 ± 86 % Air changes: approximately 10-15 changes / hour Photoperiod: 12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

In-life dates: 07 Aug 1990 – 31 Aug 1990

Mating procedure: At the end of the acclimatization period, animals judged to be in good health and meeting acceptable body weight requirements (a minimum of 220 grams) were placed in a suspended wire-mesh cage with a resident male from the same strain and source for breeding. Resident males were untreated, sexually mature rats utilized exclusively for breeding. A breeding record containing the male and female identification numbers and the dates of cohabitation was prepared. The females were approximately 12 weeks old when paired for breeding.
Positive evidence of mating was confirmed by the presence of a copulatory plug in the vagina or the presence of sperm in a vaginal smear. Each mating pair was examined daily. The day on which evidence of mating was identified was termed Day 0 of gestation and the animals were separated.

Animal assignment: The mated females were consecutively assigned in a block design to groups containing eight rats each by the following randomization procedure. The first mated female and the appropriate gestation Day 0 designation were entered on the form and the female was assigned to group 1, the second mated female was assigned to group 2, and the third to group 3, etc. This process was continued daily until eight females were placed into each group. Body weight values ranged from 213 g to 261 g on Day 0 of gestation.

Dose preparation and analysis: An appropriate amount of the test item (AMPA) was weighed for each group into tared weigh boats and transferred to a mortar. The test material was triturated with corn oil, and ground with a pestle until a slurry was obtained. The slurry was transferred to a graduated cylinder via a series of vehicle rinses. A sufficient amount of vehicle was added to attain a volume of 800 mL. The cylinder was inverted several times to ensure adequate mixing and the contents were transferred to a properly labelled storage container. The cylinder was rinsed with an additional 620 mL of vehicle, which was also added to the storage container. The preparations were then mixed for approximately 5 minutes on a Polytron PT6000 homogenizer to reduce particle size and to ensure proper mixing. The dosing preparations were visually inspected for homogeneity prior to dispensation for the first day of dose administration.
Preparations for all dose groups were made once at study start, and were stored at room temperature. A second preparation for the 150 mg/kg bw/day group was made one week later, and stored at room temperature. The preparations were stirred using a magnetic stir plate and bar each day prior to dispensing and during the dosing procedure.
Samples of the test material preparations were analysed at the Monsanto Company. The following aliquots were drawn on the first day of dose formulation: one sample (middle) from the control group (group 1) and two samples (top and bottom) from the mid-dose group (group 3) preparations to verify concentration; and three samples (top, middle, bottom) from the low dose group (group 2) and high dose group (group 4) preparations to verify concentration and homogeneity. One week later, three aliquots (top, middle and bottom) were taken from the low dose group preparation. In the last week of dosing, two aliquots (top and bottom) were taken from the low dose and high dose group preparations. For all dates, samples at room temperature were sent to the sponsor for analysis. The analytical results were reported separately (see CA 4). AMPA concentrations in corn oil dosing solutions were found to be 105 - 120 % of the target levels.

Dose administration: The dose formulations were administered orally by gavage, via a 16-gauge stainless steel gavage cannula, once daily for 10 consecutive days initiating on gestation Day 6 and continuing up to and including Day 15 of gestation. A dosage volume of 10 mL/kg bw was used for all dose levels. The control animals received corn oil on a comparable regimen of 10 mL/kg bw. Individual dosages were based on the most recent body weights.

Clinical observations: All animals were observed twice daily for moribundity and mortality. Detailed clinical observations were recorded individually from Days 0 through 20 of gestation (prior to compound administration during the dosing period). Animals were observed for signs of toxicity approximately one hour after treatment throughout the dosing period; only significant findings were recorded at the post-dosing observation period.

Bodyweight and gravid uterine weight: Maternal body weights were recorded individually on gestation Days 0, 6, 9, 12, 16 and 20. A group mean body weight was calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and also for Days 6-16 and 0-20.
Gravid uterine weight, net body weight (the Day 20 body weight minus the weight of the uterus and contents) and net body weight change (the Day 0-20 body weight change minus the weight of the uterus and contents) were presented for each gravid female at the scheduled Caesarean section.

Food consumption: Individual food consumption was recorded on Days 0, 6, 9, 12, 16 and 20 of gestation. Food intake was calculated as g/animal/day and g/kg bw/day for the corresponding body weight change intervals. On the occasions when food intake could not be measured for one of the days in a given interval, food intake was calculated using the appropriate number of days for that interval.

Terminal investigations: All maternal animals were sacrificed by carbon dioxide inhalation on gestation Day 20. The thoracic, abdominal and pelvic cavities were opened by a ventral midline incision and the contents examined. In all instances, the post mortem findings were correlated with the ante mortem comments and any abnormalities were recorded. The uterus and ovaries were excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened and the number and location of all foetuses, early and late resorptions and the total number of implantation sites were recorded. Uteri with no macroscopic evidence of nidation were opened and subsequently placed in 10 % ammonium sulphide solution for detection of early implantation loss as described by Sa1ewski.
The liver, kidneys and spleen from each dam were excised, trimmed and weighed and all findings recorded. The carcasses of the dams, including all organs, were then discarded.

Foetal observations: Each foetus was individually sexed, weighed and tagged for identification. A detailed external examination of each foetus was conducted to include, but was not limited to, the eyes, palate, and external orifices and each finding was recorded. Crown-rump measurements were recorded for late resorptions and the tissues were discarded. The sex of each foetus was verified by an internal examination. Each foetus was examined viscerally by a modification of the Stuckhardt and Poppe fresh dissection technique to include the heart and major vessels. Foetal kidneys were examined and graded for renal papillae development by a method described by Woo and Hoar.
Heads from approximately one-half of the foetuses from each female were placed in Bouin's fixative for subsequent soft-tissue examination by the Wilson sectioning technique. The heads from the remaining one-half of the foetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 95 % isopropyl alcohol. Following fixation in alcohol, each foetus was macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson. The skeletal examination was conducted utilizing low power magnification provided by a stereomicroscope. External, visceral and skeletal findings were recorded as developmental variations or malformations. The foetal developmental findings were summarized by 1) presenting the incidence of a given finding both as a percentage of the no. of foetuses and the no. of litters available for examination in the group and 2) by considering the litter as the basic unit for comparison and calculating the number of affected foetuses in a litter on a proportional basis.

Statistical analyses: All analyses were conducted using two-tailed tests for a minimum significance level of 5 % comparing each treated group to the vehicle control group. Each mean was presented with the standard deviation (SD) and the number of animals (N) used to calculate the mean. The following statistical tests were performed: Chi-square test with Yates' correction factor (foetal sex ratios); Fisher's Exact test (malformations and variations); Mann-Whitney U test (early and late resorptions, dead foetuses, post-implantation losses); One-way ANOVA with Dunnett's test (corpora lutea, total implantations, viable foetuses, foetal body weights, maternal body weights and weight changes, maternal net body weight changes and gravid uterine weights, selected organ weights); Kruskal-Wallis test (litter proportions of intrauterine data, considering the litter rather than the foetus as the experimental unit).

II. RESULTS AND DISCUSSION

A: ANALYSIS OF DOSE FORMULATIONS

The analytical results were reported separately by the Monsanto Company. AMPA concentrations in corn oil dosing solutions were found to be 105 - 120 % of the target levels.

B: CLINICAL OBSERVATIONS

All animals survived to the scheduled sacrifice on gestation Day 20. The predominant clinical findings observed during the study were mucoid faeces, hair loss and soft stool. Mucoid faeces occurred only in the AMPA treated groups and with very few exceptions, occurred only during the dosing period with an occasional incidence following the dosing period. The incidence of mucoid faeces was increased by numerical progression in the 400 and 1000 mg/kg bw/day groups; the incidence of mucoid faeces in the 150 mg/kg bw/day group was limited to three consecutive days for a single animal.
Hair loss on various body surfaces (primarily the forelimbs, hindlimbs and lateral abdominal area) and soft stool occurred similarly in the control group and the 150 mg/kg/day group, however, the incidence of each finding was slightly increased and appeared treatment-related in the 400 and 1000 mg/kg bw/day groups.
In some cases, hair loss on the forelimbs was initially noted prior to the treatment period. Hair loss on the hindlimbs and lateral abdominal area was first observed after dosing initiated. Once observed, hair loss generally persisted throughout the treatment and post-treatment periods.
Soft stool generally occurred during the treatment period with an occasional incidence following the treatment period. Other clinical findings observed during the study were infrequent in occurrence or occurred similarly in the control group. No clinical findings were observed at the l-hour post-dosing observation period.

Table B.6.8.1.1.9.3-28: AMPA: Teratogenicity study in rats ([redacted], 1991b): Summary of selected clinical findings.

	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	**150**	**400**	**1000**
No. of females	25	25	25	25
Surviving until scheduled Caesarean section	25	25	25	25

Clinical finding (no. of animals affected)				
Hair loss				
Right forelimb	1	2	6	6
Left forelimb	1	3	6	6
Right abdominal area	0	0	3	6
Left abdominal area	1	0	1	6
Right inguinal area	0	0	0	1
Left inguinal area	0	0	0	1
Left lateral abdomal area	0	0	0	1
Right hindlimb	0	0	1	5
Left hindlimb	1	1	0	6
Ventral thoracic area	0	0	0	1
Excreta				
Soft stool	15	19	22	23
Mucoid faeces	0	1	7	18

C. BODY WEIGHT

Mean body weight gains at 1000 mg/kg bw/day were similar to the control group during the initial six days of the dosing period (gestation Days 6-9 and 9-12). During gestation Days 12-16, mean body weight gain at 1000 mg/kg bw/day was slightly reduced (statistically significant at $p<0.01$) compared to the control group. Mean body weight gains at 1000 mg/kg bw/day were similar to the control group when evaluated over the entire treatment period (gestation Days 6-16) and the post-treatment period (gestation Days 16-20). Mean absolute body weight values at 1000 mg/kg bw/day were comparable to the control group throughout the study. No adverse effects on mean body weights and mean body weight gains were observed at 150 and 400 mg/kg bw/day during the study.

Mean gravid uterine weights, net body weight gains and net body weights in the AMPA treated groups were similar to the control group.

Table 6.8.1.1.9.3-29: AMPA: Teratogenicity study in rats ([redacted] 1991b): Summary of gravid female body weight, body weight gain and gravid uterine weight.

	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	**150**	**400**	**1000**
No. of females	24	24	24	24
Body weights (g ± SD) Day post coitum				
0	232 ± 11.5 (100%)	233 ± 11.1 (100%)	234 ± 10.0 (101%)	234 ± 10.7 (101%)
6	267 ± 12.2 (100%)	266 ± 13.6 (100%)	270 ± 11.2 (101%)	268 ± 13.4 (100%)
12	281 ± 14.3 (100%)	285 ± 14.5 (101%)	289 ± 12.2 (103%)	285 ± 15.2 (101%)
16	315 ± 16.1 (100%)	315 ± 16.3 (100%)	321 ± 16.0 (102%)	310 ± 15.4 (98%)
20	379 ± 19.9 (100%)	379 ± 21.1 (100%)	381 ± 23.1 (101%)	369 ± 26.4 (97%)
Body weight gain (g ± SD) Days post coitum				
6-9	2 ± 5.2 (100%)	5 ± 5.0 (250%)	4 ± 6.5 (200%)	4 ± 7.0 (200%)
9-12	13 ± 6.6 (100%)	14 ± 4.0 (108%)	15 ± 4.9 (115%)	13 ± 6.5 (100%)
12-16	33 ± 7.1 (100%)	30 ± 7.3 (91%)	32 ± 7.4 (97%)	**26**** ± 11.0 (79%)
6-16	48 ± 8.1 (100%)	49 ± 6.6 (102%)	51 ± 11.0 (106%)	43 ± 10.0 (90%)
Gravid uterine weight (g ± SD)	78.5 ± 7.53 (100%)	77.7 ±9.12 (99%)	76.9 ± 14.36 (98%)	75.7 ± 7.11 (96%)

SD = Standard deviation
** significantly different from control p<0.01 (Dunnett's test, two-tailed)

D. FOOD CONSUMPTION

Food consumption (evaluated as g/animal/day and g/kg bw/day) at 1000 mg/kg bw/day was slightly decreased compared to the control group during the initial three days of dosing (gestation Days 6-9); this was statistically significant (p<0.05) when evaluated as g/kg bw/day. During gestation Days 9-12, food intake (g/animal/day and g/kg bw/day) at 1000 mg/kg bw/day was slightly increased (p<0.05) when compared to the control group. Food consumption at 1000 mg/kg bw/day was similar to the control group for the remainder of the treatment period (gestation Days 12-16), the entire treatment interval (gestation Days 6-16), and the post-treatment period (gestation Days 16-20).

Food consumption, evaluated as g/animal/day and g/kg bw/day, was apparently unaffected by treatment at dose levels of 150 and 400 mg/kg bw/day. The only statistically significant differences were in the 400 mg/kg bw/day group; food consumption values were slightly increased (g/animal/day and g/kg bw/day) during gestation Days 12-16, 6-16, and 0-20 (p<0.05).

Table B.6.8.1.1.9.3-30: AMPA: Teratogenicity study in rats ([redacted], 1991b): Summary of gravid female consumption.

	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	**150**	**400**	**1000**
No. of females	24	24	24	24
Mean food consumption (g/kg bw/day ± SD) Days post coitum				
0-6	82 ± 6.5	83 ± 6.5	85 ± 6.3	82 ± 7.5

	(100%)	(101%)	(104%)	(100%)
6-9	52 ± 8.5 (100%)	54 ± 7.3 (104%)	52 ± 12.2 (100%)	**44*** ± 10.0 (85%)
9-12	56 ± 7.1 (100%)	56 ± 7.4 (100%)	60 ± 6.7 (107%)	**62*** ± 13.1 (111%)
12-16	61 ± 6.0 (100%)	60 ± 5.5 (98%)	**66*** ± 7.6 (108%)	60 ± 8.5 (98%)
16-20	80 ± 4.6 (100%)	80 ± 4.6 (100%)	81 ± 5.8 (101%)	81 ± 10.3 (101%)
6-16	56 ± 5.2 (100%)	57 ± 5.0 (102%)	**60*** ± 6.0 (107%)	56 ± 6.3 (100%)
0-20	68 ± 3.9 (100%)	68 ± 4.4 (100%)	**71*** ± 4.4 (104%)	68 ± 4.4 (100%)

SD = Standard deviation
* significantly different from control p<0.05 (Dunnett's test, two-tailed)

E. NECROPSY

Gross pathology: At scheduled sacrifice on gestation day 20, no treatment-related gross necropsy findings were observed. Dilated renal pelvis were observed in 2, 3 and 1 animal(s) in the control, 150 and 1000 mg/kg bw/day groups, respectively. A single animal in the control group had a white area on the spleen. There were no other macroscopic findings.

Organ weights: Mean liver, kidney and spleen weights in the treated groups were similar to the control group, indicating no apparent adverse effect from AMPA administration.

Caesarean section data:
No adverse effects were observed at any dose level on post-implantation loss, the numbers of viable foetuses, or foetal sex ratios. All values in the 150, 400 and 1000 mg/kg bw/day groups were comparable to the control group when evaluated on a group mean or proportional (%) litter basis. The mean numbers of corpora lutea and implantation sites in the AMPA treated groups were similar to the corresponding control group values.

Table 6.8.1.1.9.3-31: AMPA: Teratogenicity study in rats ([redacted], 1991 b): Intergroup comparison of maternal performance and Caesarean section data

Observation	**Dose level of AMPA (mg/kg bw/day)**			
	0 (control)	**150**	**400**	**1000**
No. of animals mated	25	25	25	25
No. of premature decedents	0	0	0	0
No. of animals pregnant	24	24	24	24
Pregnancy frequency (%)	96	96	96	96
Mean No. of corpora lutea ± SD	16.1 ± 1.45	17.0 ± 2.51	16.4 ± 1.61	16.5 ± 2.00
Mean No. of implantations ± SD	15.0 ± 1.33	15.3 ± 1.29	15.0 ± 2.40	15.1 ± 1.15
Mean post-implantation loss ± SD (%)	0.7 ± 1.05 (4.4%)	0.8 ± 0.83 (5.3%)	0.6 ± 0.88 (3.9%)	0.6 ± 0.83 (3.8%)
Mean viable foetuses ± SD (%)	14.4 ± 1.66 (95.6%)	14.5 ± 1.64 (94.7%)	14.4 ± 2.55 (96.1%)	14.5 ± 1.28 (96.2%)
Mean dead foetuses ± SD	0.0 ± 0.00 (0%)	0.0 ± 0.00 (0%)	0.0 ± 0.00 (0%)	0.0 ± 0.00 (0%)
Mean foetal weight (g) ± SD	3.5 ± 0.25 (100%)	3.5 ± 0.18 (100%)	3.4 ± 0.19 (97%)	**3.3*** ± 0.31 (94%)
Total No. of male:female foetuses	162:183	182:165	179:167	180:169

SD = Standard deviation
* significantly different from control p<0.05 (Dunnett's test, two-tailed)

F. FOETUSES

Mean foetal body weight at 1000 mg/kg bw/day was slightly and statistically significantly ($p<0.05$) decreased (3.3 g) when compared to the control group (3.5 g). This reduction was mainly attributable to two litters with mean foetal body weights of 2.4 g and 2.8 g, respectively. Mean foetal body weight at 1000 mg/kg bw/day was similar to the minimum value in the laboratory historical control data (3.3 g). A mean foetal body weight of 3.3 g has been observed in five of 85 (6 %) of the studies in the laboratory historical control data. At 150 and 400 mg/kg bw/day, mean foetal body weights were either identical to or were similar to the control group (3.5 g and 3.4 g, respectively). A mean foetal body weight of 3.4 g has been observed in 20 of 85 studies (24 %) in the laboratory historical control data set.

There was-no effect of AMPA on the number or survival of foetuses.
The numbers of foetuses (litters) available for morphological evaluation were 345 (24), 347 (24), 346 (24) and 349 (24) in the control, 150, 400 and 1000 mg/kg bw/day groups, respectively. The number of foetuses (litters) with malformations were 2 (2), 4 (4), 0 (0) and 2 (2) in these same dose groups, respectively.

External examination: External malformations were observed in the control, 150 and 1000 mg/kg bw/day groups. One foetus in the control group had microphthalmia (unilateral). At 150 mg/kg bw/day, one dam had dizygotic conceptuses. It was observed that the umbilical structures of the resorbed foetus were reduced and conjoined with the umbilical cord of the surviving foetus, and the resorbed foetus was enclosed in a sac. At the subsequent visceral and skeletal examinations, it was noted that this viable conceptus had a soft tissue malformation and variation (an interrupted aortic arch and a distended ureter, respectively) and a skeletal malformation (a vertebral anomaly without an associated rib anomaly). Detailed descriptions of these soft tissue and skeletal anomalies are presented in the table below. At 1000 mg/kg bw/day, two anomalies were observed: one foetus had localized oedema in the neck and thoracic regions, and another foetus had sternoschisis. No external developmental variations were observed at any dose level

Visceral examination: Soft tissue malformations were observed only in the control and the 150 mg/kg bw/day group. One foetus in the control group had hydrocephaly. At 150 mg/kg bw/day, one foetus had situs inversus. A viable dizygotic conceptus at 150 mg/kg/day had a soft tissue malformation and variation (an interrupted aortic arch and distended ureter, respectively) which were already described in the context of the external examination. Soft tissue variations were also observed in single foetuses in the control and the 400 mg/kg bw/day group. In the control group, one foetus had distended ureters. At 400 mg/kg bw/day, one foetus had a haemorrhagic ring around the iris. No other soft tissue malformations or variations were observed.

Skeletal examination: The only skeletal malformations were observed at 150 mg/kg bw/day group. A viable dizygotic conceptus had a skeletal anomaly (a vertebral anomaly without an associated rib anomaly, which was described in the context of the external examination. A single foetus had hemispherical enlargement of rib no. 3. Another foetus had a bent radius (bilateral) and a vertebral anomaly with an associated rib anomaly (left thoracic arch no. 11 and half of the centrum were absent; thoracic centrum no. 10 was malpositioned; and left rib nos. 10 and 11 were fused proximally). Several skeletal variations were observed in the control and treated groups. The most notable variants were sternebra(e) nos. 5 and/or 6 unossified, 14th rudimentary ribs, reduced ossification of the 13th ribs, and 7th cervical ribs; these are commonly observed variants in the laboratory historical control data. No dose-relationship was apparent in the percentage of foetuses (litters) affected in the dose groups and the variations occurred with a similar frequency in the control group. Also, the skeletal variants were well within the ranges observed in the laboratory historical control data. Other variants observed were infrequent occurrences or occurred similarly in the control group.

Summary of foetal examinations: Considering both the incidence and the expression of specific anomalies, the external, soft tissue and skeletal malformations observed in the treated groups appeared to be spontaneous in origin (2, 4, 0 and 2 foetuses in the control, 150, 400 and 1000 mg/kg bw/day groups had malformations). Also the skeletal variants which were observed in the study occurred with a similar frequency in the control group

Table 6.8.1.1.9.3-32: AMPA: Teratogenicity study in rats ([redacted] 1991b): Group incidence of foetal malformations

Observation	**Dose level of AMPA (mg/kg bw/day)**			
	0 (control)	**100**	**350**	**1000**
	Incidence of foetuses (litters)			
External examination				
No. of foetuses (litters) examined	345 (24)	347 (24)	346 (24)	349 (24)
Dizygotic conceptuses	0 (0)	1 (1)	0 (0)	0 (0)
Sternoschisis	0 (0)	0 (0)	0 (0)	1 (1)
Localized foetal oedema	0 (0)	0 (0)	0 (0)	1 (1)
Microphthalmia and/or anophthalmia	1 (1)	0 (0)	0 (0)	0 (0)
Visceral examination				
No. of foetuses (litters) examined	345 (24)	347 (24)	346 (24)	349 (24)
Interrupted aortic arch	0 (0)	1 (1)	0 (0)	0 (0)
Situs inversus	0 (0)	1 (1)	0 (0)	0 (0)
Hydrocephaly	1 (1)	0 (0)	0 (0)	0 (0)
Skeletal examination				
No. of foetuses (litters) examined	345 (24)	347 (24)	346 (24)	349 (24)
Bent limb bone(s)	0 (0)	1 (1)	0 (0)	0 (0)
Rib(s) - hemispherical enlargement	0 (0)	1 (1)	0 (0)	0 (0)
Vertebral anomaly with or without associated rib anomaly	0 (0)	2 (2)	0 (0)	0 (0)
Combined incidence of malformations	2 (2)	4 (4)	0 (0)	2 (2)

III. CONCLUSIONS

A marginal degree of maternal toxicity and a marginal degree of developmental toxicity were noted at 1000 mg/kg bw/day as clinical signs, transient reductions of maternal body weight gain and food consumption. Similar clinical findings were the only maternal response at 400 mg/kg bw/day. With this exception, the NOAEL (no observable adverse effect level) for maternal toxicity and developmental toxicity was considered to be 400 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This developmental toxicity study is considered acceptable, although some endpoints that are requested in the OECD 414 TG from 2018 are not included in this study. At 1000 mg/kg bw/day, a marginal degree of maternal and developmental toxicity was noted in form of clinical signs, transient reductions in body weight gain, food consumption and reduced foetal body weights. Clinical signs (alopecia and mucoid faeces) were also noted at 400 mg/kg bw/day. Accordingly, the NOAEL for maternal toxicity was considered to be 150 mg/kg bw/day, while the NOAEL for developmental toxicity was considered to be 400 mg/kg bw/day.

Assessment and conclusion by RMS:

Agreed with the conclusions by the applicant. Although there are some limitations to the study, it is considered acceptable. The maternal NOAEL is 150 mg/kg bw/day based on the clinical signs and the reduced body weight gain and food consumption. The developmental NOAEL is set at 400 mg/kg bw/day based on the significantly reduced foetal body weight. This conclusion is in line with the RAR (2015).

B.6.8.1.1.10. **Literature**

A literature search was conducted which retrieved four relevant papers which are summarized in the table below. Three of the these studies were on genetic toxicity and also included glyphosate and are therefore summarized elsewhere in the RAR (B.6.4).

Table B.6.8.1.1.10-1: Overview on literature found relevant for evaluation of AMPA

Annex Point	**Study**	**Study type Parameters investigated Test system**	**Substance Dose levels Exposure**	**Reliability and relevance comments**	**Result**
CA 5.8.1/031	Kwiatkowska M. *et al.*, 2020	Apoptosis induction in human peripheral blood mononuclear cells	AMPA, Purity: 98 % 0.01 to 5 mM	Refer to Vol 1	Refer to Vol 1
CA 5.8.1/048 (CA 5.4/009) #	Suárez-Larios *et al.*, 2017	Induction of DNA double strand breaks Cytotoxicity Westernblot analysis of proteins involved in DNA recombination In human peripheral blood lymphocytes	Glyphosate and AMPA, Purity: not reported 0.4 – 50 µM (information on S9 mix n.a.) 1.5 h exposure	Refer to Vol 1	Refer to Vol 1
CA 5.8.1/049 (CA 5.4/011) #	Roustan *et al.*, 2014	Photoactivation Micronucleus assay Intracellular ROS In CHO-K1 cells	Glyphosate and AMPA, Purity: not reported 0.005 – 100 µg/mL ± S9 mix 3 h exposure	Refer to Vol 1	Refer to Vol 1
CA 5.8.1/050 (CA 5.4/012) #	Mañas *et al.*, 2013	Comet assay (blood and liver) Oxidative stress (TBARs, SOD and CAT activity; liver, kidney, lung and heart) In Balb C mice	AMPA, Purity: 99 %, 100 mg/kg bw/day 14 day admin. in drinking water	Refer to Vol 1	Refer to Vol 1

#: The summary of the publication is provided in section 5.4

STUDY 1:

1. Information on the study

Data point:	CA 5.8.1/031
Report author	Kwiatkowska, M. *et al.*
Report year	2020
Report title	Evaluation of apoptotic potential of glyphosate metabolites and impurities in human peripheral blood mononuclear cells (*in vitro* study)
Document No	doi.org/10.1016/j.fct.2019.110888 E-ISSN: 1873-6351
Guidelines followed in study	None

Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (Research conducted in an academic laboratory)
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Study unreliable

2. Full summary of the study according to OECD format

The study aimed to assess the effect of glyphosate, its metabolites: aminomethylphosphonic acid (AMPA), methylphosphonic acid and its impurities: PMIDA, N-methylglyphosate, hydroxymethylphosphonic acid and bis (phosphonomethyl)amine on apoptosis induction in human peripheral blood mononuclear cells (PBMCs). PBMCs were exposed to the compounds studied at concentrations ranging from 0.01 to 5 mM for 4 h. It was observed an increase in reactive oxygen species (including hydroxyl radical) and cytosolic calcium ions levels as well as reduction of transmembrane mitochondrial potential (ΔΨm) in PBMCs exposed to the compounds examined. All substances studied changed PBMCs membrane permeability, activated caspase-8, -9, -3 and caused chromatin condensation, which showed that they were capable of inducing apoptosis both via extrinsic and particularly intrinsic pathway. Generally the study demonstrated that there were no differences between apoptotic changes induced by glyphosate, its metabolites or impurities, and observed changes were provoked by high concentrations of investigated compounds. Since clear changes were only seen at high concentrations, a low apoptotic potential of these compounds was concluded by the study authors.

I. MATERIALS AND METHODS

Chemicals; The investigated compounds i.e., aminomethylphosphonic acid (AMPA) (purity 98%), methylphosphonic acid (purity 98%), N-(phosphonomethyl)iminodiacetic acid (PMIDA) (purity 98 %), N-methylglyphosate, hydroxymethylphosphonic acid (purity 98%) and bis-(phosphonomethyl)amine (purity 97%) were synthetized by the Institute of Industrial Organic Chemistry, Warsaw, Poland. Glyphosate [N-(phosphonomethyl)glycine] (purity 95%) in acid form was bought from Sigma-Aldrich, USA. The investigated compounds were dissolved in phosphate-buffered saline (pH 7.4). Other chemicals were purchased from POCh (Poland) and Roth (Germany) and were of analytical grade. Different concentrations of glyphosate have been selected in this study:

- 0.01 mM (low concentration) that is quite similar to the concentration determined in blood of humans who were not directly exposed to this herbicide,
- 0.05 mM to 0.5 mM (moderate to high concentration) that corresponds to the concentration that may penetrate into human blood as a result of glyphosate formulation poisoning,
- 5 mM to 10 mM (very high concentration) that corresponds to the concentration detected in humans after acute poisoning with glyphosate formulation.

Cells isolation; PBMCs were isolated from leucocyte-buffy coat obtained from blood collected in Blood Bank in Lodz, Poland. Blood was taken from healthy, non-smoking volunteers who showed no signs of infection disease symptoms at the time the blood samples were collected. The study was approved by the Bioethics Committee of the University of Lodz No. KBBN-UŁ/I/3/2013. The final PBMCs density used in the experiments (after addition of glyphosate, its metabolites or impurities) was $2x10^6$ per 1 mL. The viability of the cells was over 95% as determined by flow cytometry.

Quantitative determination of apoptosis (YO-PRO-1/PI staining); Apoptosis is characterized by changes in cell membrane permeability. Apoptotic cells that have altered membrane permeability (membrane integrity is maintained) are permeable for a marker YOPRO-1 (carbocyanine nucleic acid

stain), which exhibits green fluorescence, whereas they are not permeable for the dye propidium iodide (PI) that shows red fluorescence. The cells were treated with glyphosate, its metabolites and impurities in the final concentrations ranging from 0.01 to 10 mM and incubated for 4 h at 37 °C in total darkness. Apoptosis was induced with 10 μM of camptothecin. After the incubation, the samples were centrifuged at 300 g for 5 min at 4 °C, the supernatant was removed, and the cells were supplemented with RPMI with L-glutamine and 10 % FBS. Then, the mixture of YO-PRO-1 and PI (0.1 μM each) was added to the samples, which were incubated for 20 min on ice in total darkness. The samples were analysed by flow cytometry (LSR II, Becton Dickinson) with excitation maximum at 488 nm to visualize the YO-PRO-1 green fluorescence (520/30 bandpass filter) and PI red fluorescence (610/20 bandpass filter). FMC gate on PBMCs has been established for data acquisition and the data were recorded for a total of 10,000 events per sample.

Determination of biochemical and morphological hallmarks of apoptosis

Cytosolic calcium ion level; Calcium ion accumulated in mitochondria and endoplasmatic reticulum is an important secondary messenger in controlling apoptotic cell death. The level of cytosolic calcium ions was analysed by flow cytometry (LSR II, Becton Dickinson) using a fluorescent probe Fluo-3/AM according to manufacturer's protocol. Fluo-3/AM passes through membrane of living cells, in which it is cleavaged by intracellular esterases to Fluo-3. Inside the cell, Fluo-3 exhibits green fluorescence after complexation with calcium ions. PBMCs treated with glyphosate, its metabolites or impurities in the final concentrations ranging from 0.01 to 10 mM were incubated for 4 h at 37 °C in total darkness. Next, the cells were centrifuged at 300 g for 5 min at 4 °C, suspended in Fluo-3/AM (1 μM) solution and incubated at 37 °C for 20 min in total darkness. Then, Hanks' Balanced Salt Solution (HBSS), composed of inorganic salts and supplemented with glucose (with 1 % of BSA) was added to the cells suspension, and PBMCs were incubated for 40 min at 37 °C in total darkness. The cells were centrifuged at 300 g for 5 min at 4 °C and washed twice with HEPES buffer. After centrifugation, PBMCs were suspended in HEPES buffer and incubated for 10 min at 37 °C in total darkness. The samples were analysed using flow cytometer (LSR II, Becton Dickinson) with excitation at 490/500 nm to visualize the Fluo-3 fluorescence. FMC gate on PBMCs has been established for data acquisition and the data was recorded for a total of 10,000 events per sample.

Mitochondrial transmembrane potential (ΔΨm); Mitochondrial dysfunction leading to collapse of transmembrane mitochondrial potential has been shown to participate in the induction of apoptosis. Transmembrane mitochondrial potential was shown as red fluorescence intensity of MitoTracker Red CMXRos (excitation/emission maxima – 579/599 nm). This probe is cell permeable and contains mildly thiol-reactive chloromethyl moiety for mitochondrial labeling. Nigericin and valinomycin (1 μM) were used to increase and decrease ΔΨm, respectively. PBMCs were exposed to glyphosate, its metabolites or impurities for 4 h at 37 °C in total darkness. Then, the samples were centrifuged at 300 g for 5 min at 4 °C, the supernatant was decanted, and the cells were suspended in PBS. The cells were stained with MitoTracker CMXRos in the final concentration of 1 μM for 15 min at 37 °C in total darkness, and then analysed in 96-well plates using a microplate reader (Cary Eclipse, Varian).

Caspase-8, -9 and -3 activity; Caspases are critical enzymes of apoptosis. The caspases are able to break down peptide bonds via cysteine residues in various substrates, and therefore they catalyze the apoptotic cell death irreversibly in mammals including human beings. Analysis of caspase-8 and -3 was executed according to the manufacturer's protocols. The assays were based on the hydrolysis of the peptide substrates such as acetyl-Ile-Glu-Thr-Asp-7-amino-4-methylcoumarin (Ac-IETDAMC) by caspase-8 and acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (Ac-DEVD-AMC) by caspase-3, which resulted in the release of the fluorescent 7-amino-4-methylcoumarin (AMC). The excitation and emission wavelengths of AMC were 360 nm and 460 nm, respectively. Caspase-9 colorimetric assay was based on hydrolysis of the substrate acetyl-Leu-Glu-His-Asp-p-nitroaniline (Ac-LEHD-pNA), which led to the release of p-nitroaniline (pNA) that absorbance was determined at 405 nm. Camptothecin (10 μM) was used to induce apoptosis. Detection of caspase-3 and -8 activities was executed using fluorescent microplate reader (Fluoroskan Ascent FL, Labsystem) and determination of

caspase-9 activity was performed using absorbance microplate reader (BioTek ELx808, Bio-Tek).

Hoechst 33342/PI staining; Chromatin condensation paralleled by DNA fragmentation is one of the most important criteria, which are used to identify apoptotic cells. Morphological changes of chromatin in PBMCs were assessed by double staining with Hoechst 33342 and PI. The cells were exposed to glyphosate, its metabolites or impurities for 4 h at 37 °C in total darkness. After incubation, PBMCs were centrifuged at 200 g for 3 min at 4 °C. The supernatant was removed, and the cells were suspended in PBS (0.5 ml). Then the mixture of 1 µl of Hoechst 33342 and 1 µl of PI (1 mg/ml each) was added. After 1 min incubation at 37 °C in total darkness, the cells were analysed by fluorescence microscope (Olympus IX70, Japan) at 400x magnification. PMBCs were classified on the basis of their morphological staining characteristics: viable (blue fluorescence), early apoptotic (intensive bright blue fluorescence), late apoptotic (blue-violet fluorescence) and necrotic (red fluorescence).

Determination of reactive oxygen species level

Oxidation of 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate; ROS play a central role in regulation of the main pathways of apoptosis mediated by mitochondria, death receptors and the endoplasmic reticulum (ER). The rate of 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (H2DCF-DA) oxidation was assessed by flow cytometry. 6-CarboxyH2DCF-DA is a compound widely used for the detection of intracellular oxidants production. The probe diffuses across cellular membrane where it is hydrolyzed by intracellular esterases to 6-carboxy-2′,7'dichlorodihydrofluorescein (6-carboxy-H2DCF) that after oxidation, yields highly fluorescent 6-carboxy-2′,7′-dichlorofluorescein (DCF). The cells were treated with glyphosate, its metabolites or impurities for 4 h at 37 °C in total darkness. Next, fluorescent marker was added to PBMCs, which were stained for 15 min at 37 °C in the dark. The final concentration of the fluorescent probe was 10 µM. Positive control consisted of hydrogen peroxide (2 mM). FMC gate on PBMCs has been established for data acquisition, and fluorescence was measured with the excitation and emission maxima of 488 and 530 nm, respectively. The data was recorded for a total 10,000 events per sample.

Oxidation of hydroxyphenyl fluorosceine; Highly reactive oxygen species (mainly hydroxyl radical) were assessed using flow cytometer (Becton Dickinson, LSR II) and 3-(p-hydroxyphenyl)-fluorescein (HPF). HPF is nonfluorescent until it reacts with hydroxyl radical. As a result of oxidation, the probe exhibits bright green fluorescence (excitation/emission maxima – 490/515 nm). Formation of hydroxyl radical was provoked by the addition of the mixture of ferrous perchlorate(II) (0.1 mM) and hydrogen peroxide (1 mM) to PBMCs suspension. Finally, the cells were treated with HPF in the final concentration of 2 µM and incubated for 15 min at 37 °C in total darkness. The data was recorded for a total of 10,000 events per sample.

Statistical analysis; The statistical analysis was performed with STATISTICA 8 data analysis software (2000 StatSoft, Inc., Tulsa, OK, USA). In this study, one-way analysis of variance (ANOVA) (p and F added to the description of the results) with post hoc multiple comparisons procedure (Tukey test) was used to assess statistical differences in case of normal distribution (significance marked on the charts). The difference was considered to be significant for $P < 0.05$. The individual analysis was performed on blood from 4 to 5 donors, while each experiment was repeated trice.

II. RESULTS

Apoptotic changes; After 4 h incubation, all compounds studied caused an increase in the number of apoptotic cells. Apoptotic changes were observed in PBMCs treated with 0.5 mM and 5 mM of glyphosate, PMIDA and hydroxymethylphosphonic acid. Two glyphosate metabolites: AMPA and methylphosphonic acid as well as two glyphosate impurities: N-methylglyphosate and bis-(phosphonomethyl)amine caused apoptotic changes only at their highest concentration of 5 mM (Fig. 1).

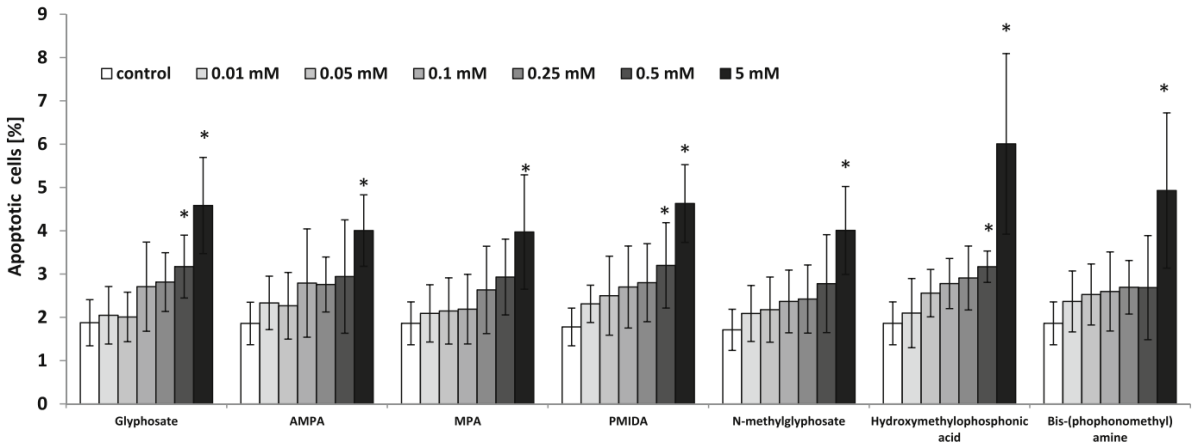


Fig. 1. Changes in the number of apoptotic PBMCs (expressed in per cent) after 4 h incubation with glyphosate, its metabolites and impurities. (*) Statistically significant changes compared to the control ($P < 0.05$).

Morphological changes of chromatin; Cell staining with Hoechst 33342 and PI allowed for the observation of early apoptotic cells (blue color), late apoptotic cells (blue-violet color) and necrotic cells (red color) in the population of PBMCs exposed to the compounds examined for 4 h. Selected photographs showing the presence of individual types of apoptotic and necrotic PMBCs are presented below (Photo 1).

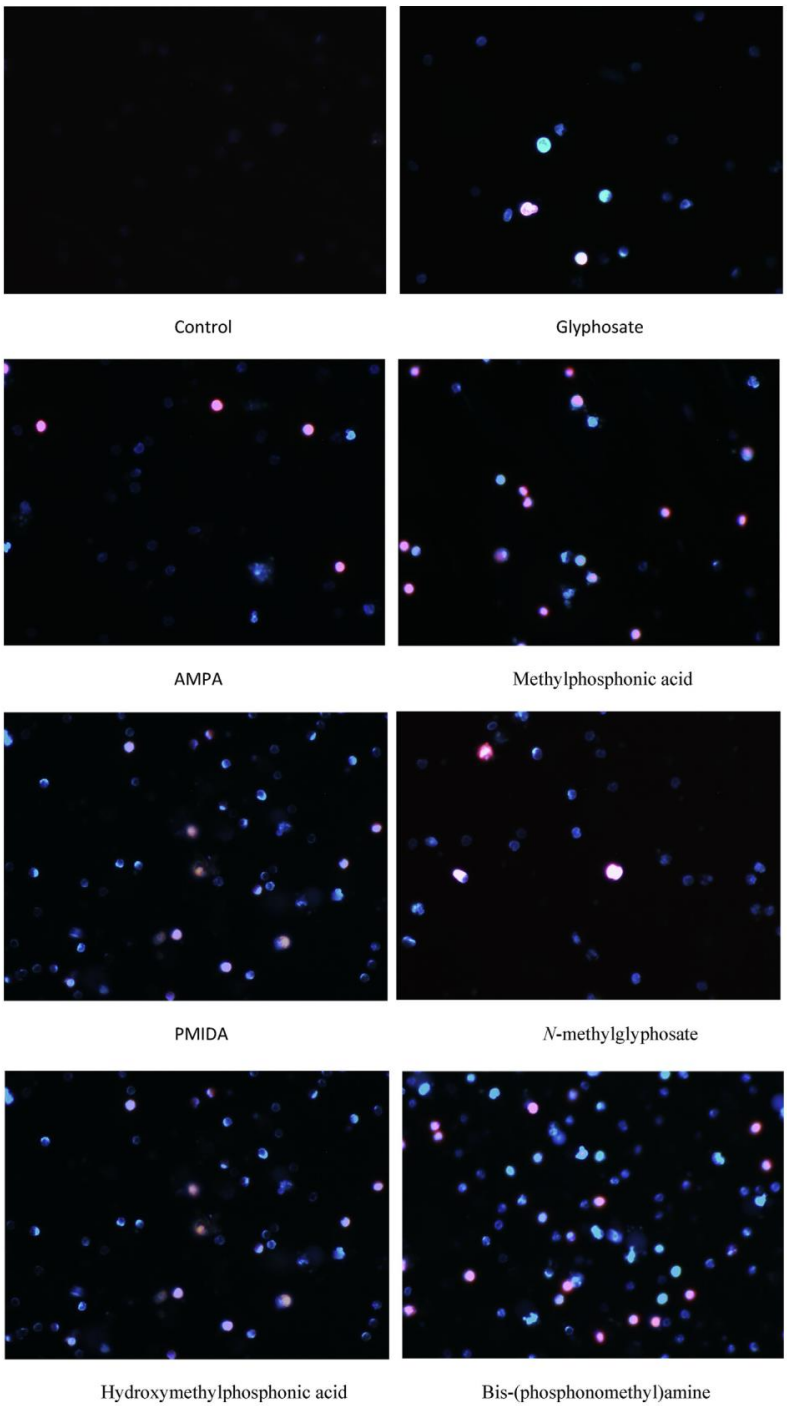


Photo 1. The representative photomicrographs of Hoechst 33324/PI-stained PBMCs pretreated with PBS (control) and with 5 mM of glyphosate, AMPA, methylphosphonic acid, PMIDA, *N*-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine. Viable cells (blue fluorescence), early apoptotic cells (intensive bright blue fluorescence), late apoptotic cells (blue-violet fluorescence) and necrotic cells (red fluorescence).

Cytosolic calcium ion level; A statistically significant increase in cytosolic calcium ion level was observed after incubation of PBMCs with glyphosate and other compounds studied (except for PMIDA). Statistically significant changes were observed for the highest concentration (5 mM) of glyphosate, AMPA and N-methylglyphosate. Glyphosate metabolite - methylphosphonic acid and glyphosate impurity - bis(phosphonomethyl)amine from the concentration of 0.5 mM caused an increase in cytosolic calcium ion level, while hydroxymethylphosphonic acid induced changes in the parameter studied from the concentration of 0.25 mM (Fig. 2).

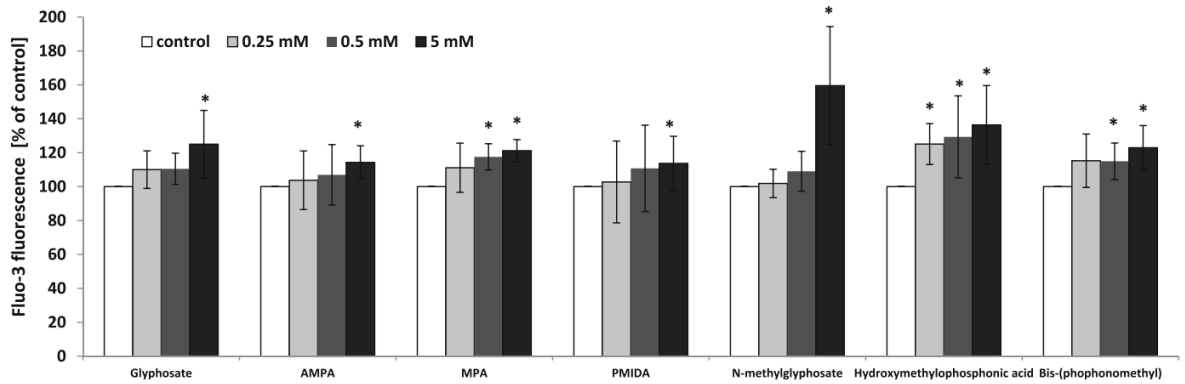


Fig. 2. Changes in cytosolic calcium ion level in control PBMCs and PBMCs incubated with glyphosate, its metabolites and impurities in the concentrations ranging from 0.25 to 5 mM for 4 h (*) Statistically significant changes compared to control ($P < 0.05$).

Transmembrane mitochondrial potential (ΔΨm); Most of the compounds studied caused a decrease in transmembrane mitochondrial potential, while hydroxymethylphosphonic acid (at 0.25 mM) caused statistically significant increase in the parameter examined. Glyphosate and one of its production impurities: bis(phosphonomethyl)amine (from the concentration of 0.05 mM) decreased ΔΨm. Other glyphosate metabolites: AMPA and methylphosphonic acid, and two glyphosate impurities: PMIDA and N-methylglyphosate decreased the parameter studied from the concentration of 0.1 mM (Fig. 3).

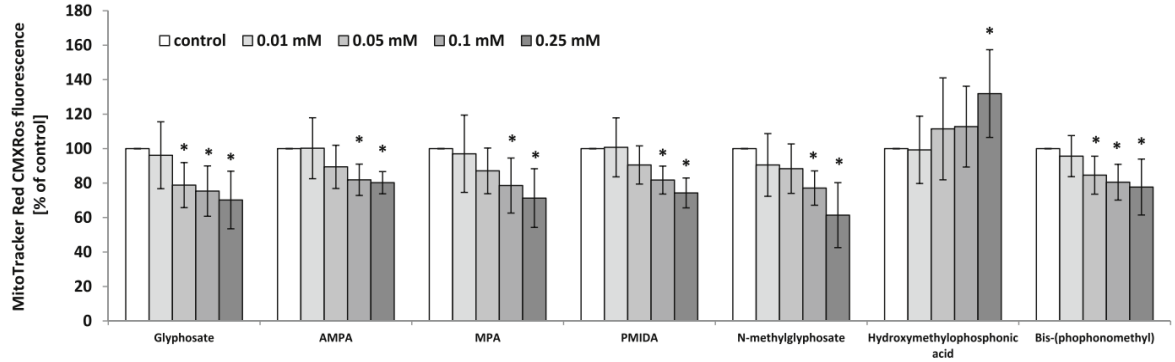


Fig. 3. Changes in transmembrane mitochondrial potential in control PBMCs and PBMCs incubated for 4 h with glyphosate, its metabolites and impurities in the concentrations ranging from 0.01 to 0.25 mM (*) Statistically significant changes compared to control ($P < 0.05$).

Caspase-8 and -9 activity; Glyphosate, AMPA, PMIDA and bis-(phosphonomethyl)amine increased in caspase-8 activity. The changes were observed in PBMCs treated with 0.5 mM of glyphosate and AMPA and with the highest concentration (5 mM) of PMIDA and bis-(phosphonomethyl)amine. Other compounds studied such as methylphosphonic acid, N-methylglyphosate and hydroxymethylphosphonic acid did not cause statistically significant changes in caspase-8 activity (Fig. 4). Glyphosate, its metabolites and impurities caused a substantial increase in caspase-9 activity. Changes in the enzyme activity were observed for the highest concentration (5 mM) of glyphosate, its two metabolites: AMPA and methylphosphonic acid and all glyphosate impurities: PMIDA, N-methylglyphosate, hydroxymethylphosphonic acid and bis(phosphonomethyl)amine (Fig. 5).

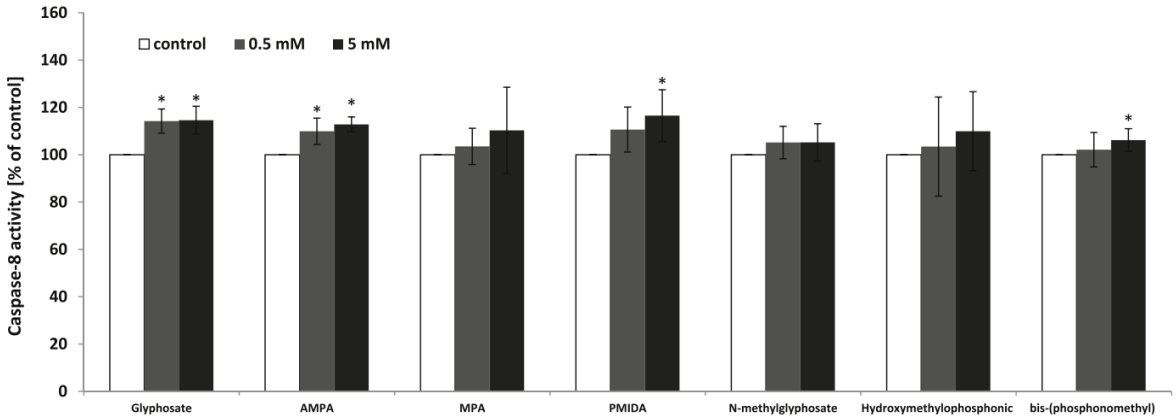


Fig. 4. Changes in caspase-8 activity in PBMCs after 4 h incubation with glyphosate, its metabolites and impurities. (*) Statistically significant changes compared to control ($P < 0.05$).

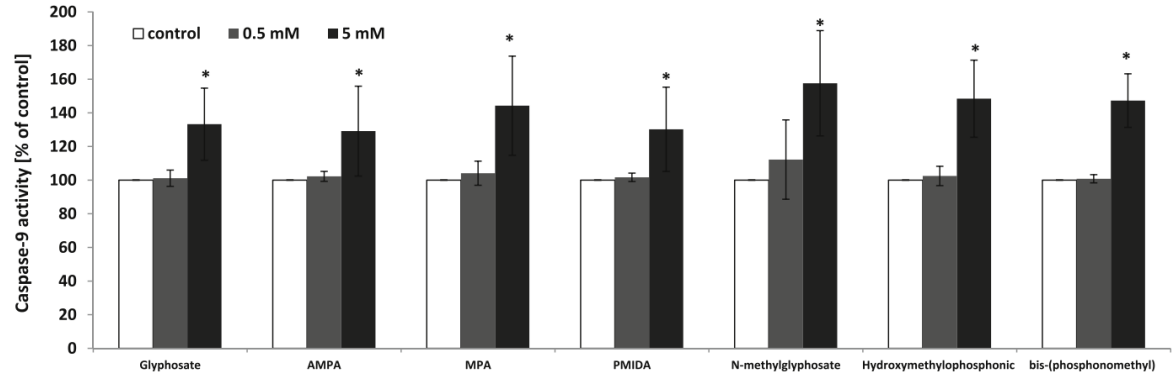


Fig. 5. Changes in caspase-9 activity in PBMCs after 4 h incubation with glyphosate, its metabolites and impurities. (*) Statistically significant changes compared to control ($P < 0.05$).

Caspase-3 activity; An increase in caspase-3 activity was noted in PBMCs treated with 0.25 mM of glyphosate and PMIDA and with 0.5 mM of AMPA, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine. Other compounds tested: N-methylglyphosate and methylphosphonic acid only at the highest concentration of 5 mM caused an increase in caspase-3 activity (Fig. 6).

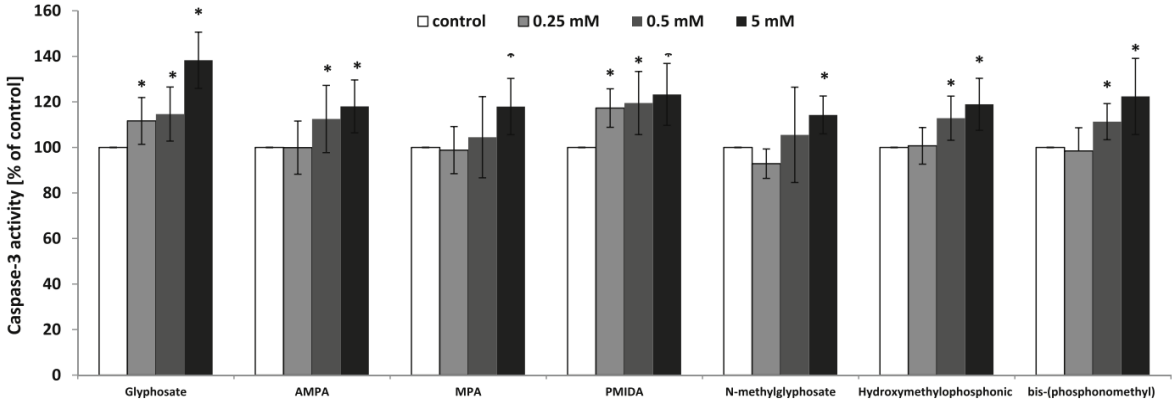


Fig. 6. Changes in caspase-3 activity in PBMCs after 4 h incubation with glyphosate, its metabolites and impurities. (*) Statistically significant changes compared to control ($P < 0.05$).

ROS level; It was observed that glyphosate, its metabolites and impurities induced a statistically significant increase in H2DCFDA oxidation in PBMCs. Changes in ROS level were observed after 4 h of exposure of PBMCs to 0.25 mM of glyphosate, methylphosphonic acid, PMIDA, N-methylglyphosate and hydroxymethylphosphonic acid. Other compounds studied like AMPA and bis-(phosphonomethyl)amine from the concentration of 0.5 mM caused an increase in the H2DCFDA

oxidation (Fig. 7).

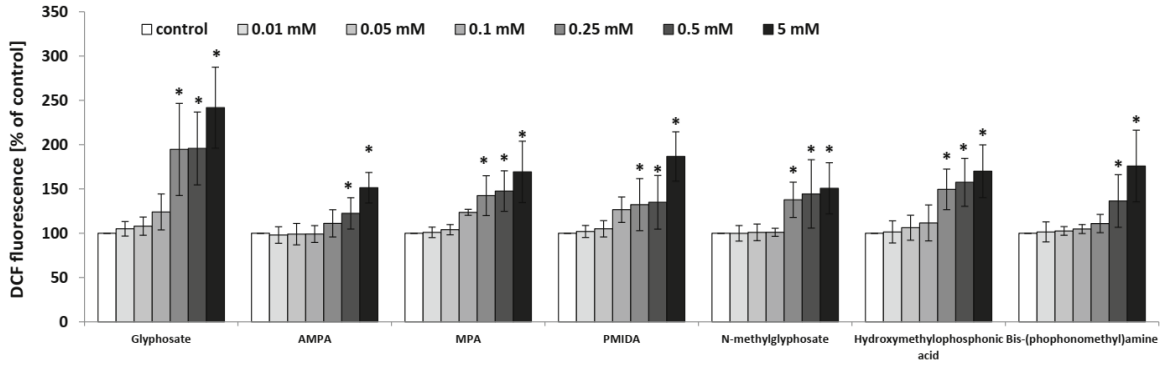


Fig. 7. Changes in total ROS level in PMBCs incubated with glyphosate, its metabolites and impurities for 4 h (*) Statistically significant changes compared to the control ($P < 0.05$).

Hydroxyl radical level; Glyphosate, its metabolites and impurities increased highly reactive oxygen species level, including hydroxyl radicals in PMBCs. Statistically significant changes were observed for most of the compounds studied from the concentration of 5 mM. An increase in HPF fluorescence was noted in cells treated for 4 h with the highest concentration (5 mM) of glyphosate, its impurities: N-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine and metabolites: AMPA and methylphosphonic acid. The strongest changes in HPF oxidation were caused by PMIDA, which changed this parameter from the concentration of 0.5 mM (Fig. 8).

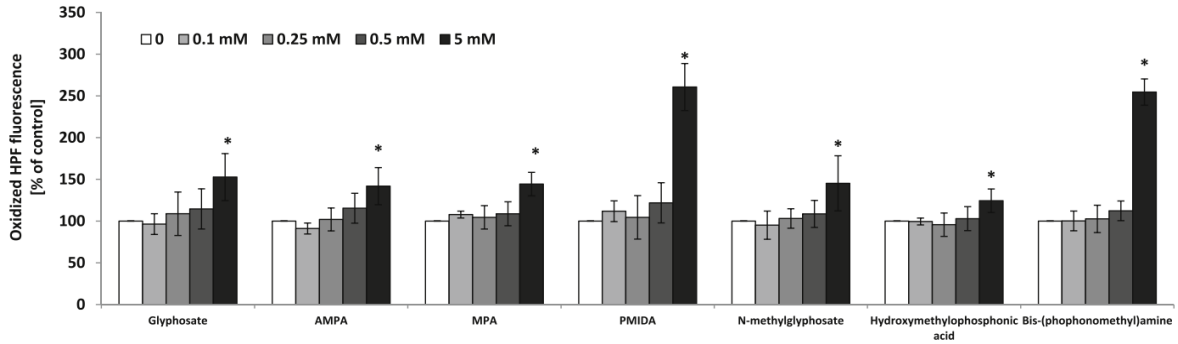


Fig. 8. The level of highly reactive oxygen species (mainly hydroxyl radical) in human PMBCs incubated with glyphosate, its metabolites and impurities for 4 h (*) Statistically significant changes compared to the control ($P < 0.05$).

Discussion

Apoptotic potential of glyphosate has been evaluated in various cell types; however, the effect of glyphosate, its metabolites (including AMPA and methylphosphonic acid) and its impurities on apoptotic changes in human leucocytes has not been assessed. This study has described apoptotic potential of glyphosate, its metabolites and impurities in human PBMCs. Besides a quantitative determination of apoptotic cells (staining with YO-PRO-1/PI fluorescent probes) the analysis concerned evaluation of the mechanism of action of these substances by measurement of a variety of parameters involved in the programmed cell death. The activities of caspases, of both initiator caspase-8 and -9, as well as executor caspase-3 were determined. Alterations in cytosolic calcium ion and ROS levels were also analysed. Moreover, changes in transmembrane mitochondrial potential and chromatin condensation were assessed. PBMCs were exposed to tested compounds for 4 h, the time necessary to observe apoptotic changes.

Flow cytometry analysis has demonstrated a statistically significant increase in the number of apoptotic cells exposed to all compounds studied. Apoptotic changes induced by glyphosate, PMIDA and hydroxymethylphosphonic acid were observed from the concentration of 0.5 mM, while those induced by AMPA, methylphosphonic acid, N-methylglyphosate and bis-(phosphonomethyl)amine

from the concentration of 5 mM. A slight increase in the number of apoptotic cells (5.76 %) treated with glyphosate was observed, compared to the control (1.22 %).

It was observed that methylphosphonic acid at lower concentration (0.25 mM) (in comparison to other analysed compounds) caused a statistically significant increase of cytosolic calcium ion level in PBMCs. Other compounds such as methylphosphonic acid and bis(phosphonomethyl)amine induced apoptosis from the concentration of 0.5 mM, while glyphosate, AMPA and N-methylglyphosate from 5 mM. It was also observed that aminomethylphosphonic acid did not cause any statistically significant increase in this parameter.

Our study demonstrated that all compounds analysed caused reduction of transmembrane mitochondrial potential. Glyphosate and its impurity - bis(phosphonomethyl)amine, from the concentration of 0.05 mM, caused reduction of $\Delta\Psi m$, while other compounds analysed induced the same changes from the concentration of 0.1 mM (except for hydroxymethylphosphonic acid, that caused reduction of the discussed parameter at the concentration of 0.25 mM).
An increased ROS production was observed in PBMCs exposed with all analysed compounds. The obtained results are consistent with data published, which observed ROS formation in human PBMCs treated with glyphosate, AMPA and particularly glyphosate preparation - Roundup 360 PLUS. It has been shown that the increase in ROS level, and hydroxyl radical in particular may contribute to DNA damage, and thus apoptotic cell death. For that reason, the formation of highly reactive oxygen species (including hydroxyl radical) was assessed in PBMCs treated with glyphosate, its metabolites and impurities. It has been noted that glyphosate, its impurities: N-methylglyphosate, hydroxymethylphosphonic acid and bis(phosphonomethyl)amine as well as its metabolites: AMPA and methylphosphonic acid at the concentration of 5 mM caused an increase in hydroxyl radical level. The highest increase in this parameter was noted in PBMCs exposed to PMIDA (from 0.5 mM Similarly, our study showed that AMPA at lower concentration did not induce hydroxyl radical formation, and the increase in discussing parameter was noted only at its highest level of 5 mM. The proteolytic activity of caspases involves cleavage of their substrates at aspartate residues. In this study we have observed that glyphosate, its metabolites and impurities caused chromatin condensation in human PBMCs.

Based on our own research, and also on studies of other authors it may be suggested that glyphosate, its metabolites and impurities induce apoptosis both via the intrinsic pathway (evidenced by the observed increase in total ROS and hydroxyl radical levels, a decrease in transmembrane mitochondrial potential and an increase in caspase-9 activity), and - to a lesser extent via the extrinsic pathway (evidenced by changes in caspase-8 activity) (except for methylphosphonic acid, Nmethylglyphosate and hydroxymethylphosphonic acid).

III. CONCLUSION

The results obtained in this study do not indicate an essential role of metabolites and production impurities of glyphosate in toxic (proapoptotic) action of glyphosate and possibly glyphosate-based preparations. Research presented in this paper indicated that both AMPA and MPA exerted a smaller effect on ROS and hydroxyl radical formation than glyphosate. For other analysed parameters, no significant differences have been demonstrated between glyphosate and its metabolites activities. Studies on toxicity of glyphosate metabolite - MPA - are even more limited, while our results clearly demonstrated a relatively low toxicity of this compound. The study also revealed that some of glyphosate impurities were characterized by a slightly stronger proapoptotic potential than the parent compound. They constitute, however, a minor impurities of glyphosate and should not significantly increase toxicity of N-(phosphonomethyl)glycine-based products (contrary to surfactants).

Obtained results clearly indicate low proapoptotic properties of all analysed compounds. Initial clear apoptotic effects are associated with their highest analysed concentrations, which correspond to the concentrations to which a human organism could be exposed only as a result of acute or subacute

poisoning with glyphosate.

Assessment and conclusion

Assessment and conclusion by applicant:

The study describes in-vitro investigations of glyphosate, its metabolites (AMPA and methylphosphonic acid) and impurities (PMIDA, N-methylglyphosate, hydroxymethylphosphonic acid, and bis-(phosphonomethyl)amine) on six intermediate endpoints of apoptosis (membrane permeability, cytosolic calcium concentration, mitochondrial transmembrane potential, caspase activity, chromatin condensation, and ROS quantitation by two methods) in human peripheral blood mononuclear cells. The reason for selection of this model is not stated but is possibly as potential target tissue for Non-Hodgkins Lymphoma. The methodologies used are frequently reported in literature but are not a standardized or validated method by GLP standards; there is no OECD guideline. Positive control results are not presented and it is unclear if positive controls were used for all assays; wording is sufficiently poor that it may be inferred that some positive controls were used, e.g. nigericin and valinomycin in the studies of mitochondrial transmembrane potential, campothecin in the caspase assays, although these may alternatively be reagents for the assay. It is unclear if assays were conducted in duplicate or triplicate (the stated term was "trice" which may be either twice or thrice), which may then also influence statistical evaluation. However, the methodology appears basically sound.

Apoptotic or pre-apoptotic activity was seen generally consistently across the assays. While glyphosate, its metabolites, and impurities were seen to increase apoptotic endpoints in these assays (0.5 mM and higher), clear effects occurred only at high concentrations.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no proper cytotoxicity tests were performed, no positive controls were used and the concentration range at which most of the effects were observed is beyond the acceptable physiological range (> 1 mM). The concentration range at which the glyphosate impurities were tested is the same as that for glyphosate which is not a realistic approach for risk assessment of impurities.

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

Publication: Kwiatkowska *et al*., 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity (95 %). Source: Sigma-Aldrich, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also glyphosate impurities were tested.
AMPA is the tested substance	Y	
Study		
Test system clearly and completely described	Y	PBMCs
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Partly	0.01, 0.05, 0.25, 0.5, 5, 10 mM
Cytotoxicity tests reported	?	
Positive and negative controls	N	No positive controls.
Complete reporting of effects observed	Y	

Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no proper cytotoxicity tests were performed, no positive controls were used and the concentration range at which most of the effects were observed is beyond the acceptable physiological range (> 1 mM). The concentration range at which the glyphosate impurities were tested is the same as that for glyphosate which is not a realistic approach for risk assessment of impurities.		

Assessment and conclusion by RMS:
As also noted by the applicant there are some limitations to the study. First the materials and methods are not entirely clear. E.g. the study mentions that apoptosis was induced with 10 µM of camptothecin but no results are presented and it is considered unlikely that this compound would be used in combination with the test compounds as this would interfere with the endpoint investigated. The same applies to nigericin and valinomycin which were reported to be used to increase and decrease ΔΨm.
Moreover, the authors report that each experiment was conducted trice which we assume is a typo, but from the publication it cannot be derived whether each experiment was run two or three times.

Based on these uncertainties the study is concluded to be unreliable. Overall, the study does not indicate a concern for AMPA as apoptotic effect were only observed at high concentrations.

B.6.8.1.1.11. QSAR and read-across

Two studies were submitted in the residue section (KCA 6.7.1-001 and KCA 6.7.1-002) for which the RMS considers that the summaries should be reported in the human health section as they concern QSAR analysis of seven metabolites.

STUDY 1

1. Information on the study

Data point	CA 6.7.1/001 >> CA 6.8.1
Report author	knoell Germany GmbH
Report year	2020
Report title	(Q)SAR and read-across genotoxicity evaluation of Glyphosate and eight metabolites, using VEGA v1.1.5b22, DEREK Nexus v6.0.1, Toxtree v3.1.0 and OECD QSAR Toolbox v4.4
Report No	110054/QSAR/1
Guidelines followed in study	Not applicable
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable

Data point	CA 6.7.1/002 >> CA 6.8.1
Report author	knoell Germany GmbH
Report year	2020
Report title	Supplementary information for (Q)SAR and read-across genotoxicity evaluation of Glyphosate and seven metabolites, using VEGA v1.1.5b22, DEREK Nexus v6.0.1, Toxtree v3.1.0 and OECD QSAR Toolbox v4.4
Report No	110054/QSAR/1
Guidelines followed in study	Not applicable
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable

Acceptability/Reliability	GRG conclusion: Valid, Category 1 AGG conclusion: Reliable with restrictions. The QSAR analysis is considered reliable for the analysis of structural alerts for genotoxicity. For the read-across analysis no suitable grouping could be derived based on the thresholds suggested by Benigni et al. 2019 and therefore less stringent thresholds were applied by the study authors. The RMS considers that the thresholds applied allow for quite a lot of structural differences and does not consider the approach to be acceptable.

QSAR analysis

Models used:
To maximise the sensitivity and specificity of the prediction, two independent (Q)SAR models were chosen based on different training sets and/or algorithms (as knowledge-based and statistical-based models) to evaluate each genotoxicity endpoint. For gene mutation, the Mutagenicity (Ames test) model (CAESAR) v2.1.13 and the Mutagenicity (Ames test) model (SarPy/IRFMN) (version 1.0.7) (as implemented in VEGA v1.1.5b22) were combined. The combined results were then compared with the prediction of the expert system DEREK Nexus mutagenicity model for prediction of gene mutation v6.0.1 (as implemented in Lhasa Nexus v2.2). In the same way, two independent models were also used to evaluate chromosome aberration, i.e. DEREK Nexus chromosome damage model for prediction of chromosomal aberrations v6.0.1 (as implemented in Lhasa Nexus v2.2) as well as Structure Alerts for the in vivo micronucleus assay in rodents (as implemented in Toxtree v3.1.0). The scientific validity of all models used in the assessment is fully documented and in line with the "OECD principles for the Validation, for Regulatory Purpose, of (Q)SAR Models".

Full details on the model used including information about modelled endpoints, used training set, information on the algorithm used for deriving the model and the molecular descriptors and information about the applicability domain were provided in the study report. Glyphosate and metabolites M02 to M09 were tested (refer to the read-across section below for an overview of the metabolites)

Results:
Only one structural alert (hacceptor-path3-hacceptor) had been identified in the in vivo micronucleus model by Toxtree v3.1.0. in all metabolites except methylphosphonic acid (M08). This alert represents a molecular framework that could account for non-covalent interactions with proteins or DNA, however only a low positive predictive value of 34% had been assigned by the developers of the model (Benigni *et al.*, 2010) to the this type of alert. The VEGA combined model (CAESAR + SarPy/IRFMN) for gene mutation and DEREK Nexus for gene mutation and chromosomal aberration did not lead to alerts. Both glyphosate and AMPA for which adequate genotoxicity data are available had this alerts while the guideline studies show that these compounds are not genotoxic.

The reliability with VEGA was moderate for all molecules except M05 and M06 for which only a low reliability could be assessed.

The overall conclusion is that the QSAR assessment does not lead to a concern for genotoxicity for any of the metabolites.

Read-across prediction of genotoxicity

Method:
In the second step, a read-across analysis was set up, considering the requirements and suggestions implemented within the EFSA guidance and the expert statements by Benigni *et al.* (2019).

The software used was the OECD QSAR Toolbox v4.4.. Several profilers are available in the OECD QSAR Toolbox (v4.4) which include structural alerts relevant for these types of interactions:

- General mechanistic profilers: DNA binding by OASIS v1.7, DNA binding by OECD v2.3, protein binding by OASIS v2.7 and protein binding by OECD v2.3;
- Endpoint specific profilers: DNA alerts for AMES, CA and MNT by OASIS v2.8, in vitro mutagenicity (Ames test) alerts by ISS v2.5, in vivo mutagenicity (Micronucleus) alerts by ISS v2.5, and protein binding alerts for chromosomal aberrations by OASIS v1.6.

Based on the thresholds suggested by Benigni et al. (2019), no suitable grouping or pairwise read-across could be suggested. Therefore, a set of less stringent thresholds was implemented in parallel (Table 6)

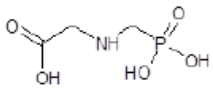
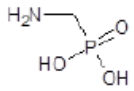
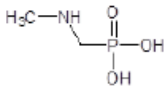
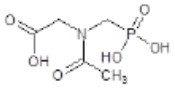
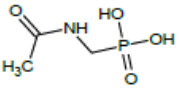
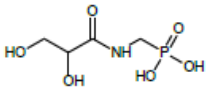
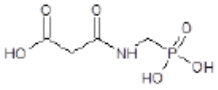
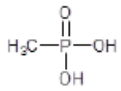
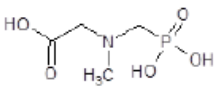
Table 6: Thresholds on parameters for simple 1:1 read-across according to Benigni and less stringent approach.

Parameter	Threshold (Benigni)	Threshold (less stringent)
Molecular Weight	Max. difference: ± 20%	Max. difference: ± 30%
Log Pow	Max. difference: ± 1 unit	Max. difference: ± 1.5 unit
Structural Similarity *	At least 70%	At least 60%

* Dice / Atom Centered Structural Similarity, calculated with the OCED QSAR Toolbox.

Glyphosate and its eight metabolites were organized into four groups, within which read-across strategies were implemented in order to fill data gaps for gene mutation and chromosome aberration.

Table 2: Details on the molecules selected for the (Q)SAR / read-across genotoxicity evaluation.

ID Substance name CAS no.	Structure	SMILES notation	MW [g/mol]	Log Kow *	Genotox evaluation based on available exp. data **
Parent (active substance)					
Parent Glyphosate 1071-83-6		OC(=O)CNCP(=O)(O)O	169	-3.4 (exp.) -4.77 (calc.)	GM negative sCA negative nCA negative
Metabolites					
M02 AMPA 1066-51-9		NCP(=O)(O)O	111	-2.47	GM negative sCA negative nCA negative
M03 N-methyl-AMPA n.a.		CNCP(=O)(O)O	125	-2	
M04 N-acetyl-glyphosate n.a.		OC(=O)CN(CP(=O)(O)O)C(C)=O	211	-2.64	GM negative sCA negative nCA negative
M05 N-acetyl-AMPA n.a.		CC(=O)NCP(=O)(O)O	153	-2.53	GM negative sCA negative nCA negative
M06 N-glyceryl-AMPA n.a.		O=C(NCP(=O)(O)O)C(O)CO	199	-3.43	
M07 N-malonyl-AMPA n.a.		O=C(CC(=O)O)NCP(=O)(O)O	197	-3.27	
M08 Methyl phosphonic acid n.a.		CP(=O)(O)O	96	-1	
M09 N-methyl-Glyphosate 24569-83-3		CN(CC(=O)O)CP(=O)(O)O	183	-4.56	GM negative

* Calculated with EPI Suite KOWWIN v1.69, as implemented in OECD QSAR Toolbox v4.4. The parent substance is part of the model's dataset and an experimental value was available.
** GM = gene mutation; sCA = structural chromosome aberration; nCA = numerical chromosome aberration

A scoring system was also implied, to summarize the overall pairwise similarities:

- One point was assigned to each parameter fulfilling the Benigni threshold
- 0.5 points were assigned to each parameter fulfilling the less stringent threshold
- 0 points otherwise

The study authors considered a score of 1.5 being the minimum score to consider two molecules as suitable for read-across. The RMS however does not agree with this approach as it allows quite a lot of structural differences.

In case of differences in the functional group profiles, the impact of specific functional groups was further investigated. Molecules sharing the functional groups under investigation were retrieved from the "Genotoxicity pesticides EFSA" database implemented in the OECD QSAR Toolbox (v4.4). The retrieved molecules were

compared with those under assessment, focusing on the structural alerts retrieved by the Toolbox's endpoint specific profilers. Finally, the available experimental data for genotoxicity was retrieved from the EFSA database, and it was statistically investigated if the functional groups could have an impact of the genotoxicity of the substances.

Results:
The results of the read-across analysis is provided in the Table below:

Table 7: Results of the simple 1:1 comparison for read-across. A blue-based colour code is used to reflect the similarity score: white for scores below 1.5, from pale to dark blue to represent increasing similarity scores (from 1.5 to 3). Complete experimental data for (negative) genotoxicity is available for parent and metabolites M02, M04 and M05; these substances have been highlighted in dark green. For M09, only experimental (negative) gene mutation data is available; this substance has been highlighted in light green.

	Parent	**M02**	**M03**	**M04**	**M05**	**M06**	**M07**	**M08**	M09
Parent Glyphosate		0.5	1	1	1.5	2	2.5	0	2.5
M02 AMPA	0.5		2.5	1	2	1	1	2	0
M03 N-methyl-AMPA	1	2.5		1	2	1	1	2	0.5
M04 N-acetyl-glyphosate	1	1	1		2	2	2.5	0	2
M05 N-acetyl-AMPA	1.5	2	2	2		2	2	0	1.5
M06 N-glyceryl-AMPA	2	1	1	2	2		2.5	0	1.5
M07 N-malonyl-AMPA	2.5	1	1	2.5	2	2.5		0	2
M08 Methyl-phosphonic-acid	0	2	2	0	0	0	0		0
M09 N-methyl-glyphosate	2.5	0	0.5	2	1.5	1.5	2	0	

As already explained no suitable read-across pairs could be identified using the strategy proposed by Benigni et al. (2019). However the authors argued that the result show a cluster of similar molecules: metabolites M04, M05, M06 and M07. A second cluster would be M02, M03 and M08. Finally the parent substance was proposed to cover metabolite M09.

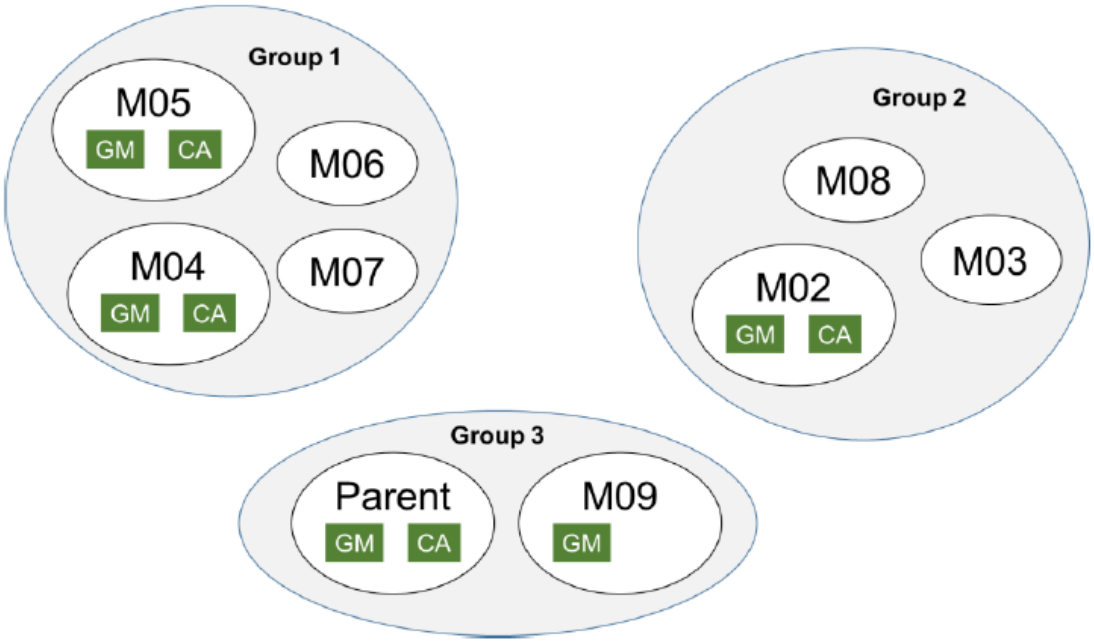


Figure 2: Grouping approach after preliminary similarity evaluation (simple 1:1 RA). A green colour code is used to represent the experimental data availability for gene mutation (GM) and chromosome aberration (CA).

The outcome of the grouping and genotoxicity evaluation strategies as reported in the study report are summarized in Table . The proposed grouping intends to support the reliability of negative predictions obtained for the metabolites for which no data is available.

According to the study authors the available experimental results show that glyphosate, AMPA (M02), *N*-acetyl glyphosate (M04), *N*-acetyl AMPA (M05) and *N*-methyl glyphosate (M09) do not exert gene mutation potential. However, the RMS notes that only for glyphosate and AMPA (M02) sufficient experimental information is available on genotoxicity and that data gaps have been set for N-acetyl AMPA (M05) and N-acetyl glyphosate (M04) to address aneugenicity. **The applicant mentions that experimental genotoxicity data is available for *N*-methyl glyphosate (M09). The applicant is requested to submit the data as these were not included in the dossier.**

No experimental data for gene mutation were available for *N*-methyl AMPA (M03), *N*-glyceryl AMPA (M06), *N*-malonyl AMPA (M07) and methyl phosphonic acid (M08).

For M03 and M08 the authors propose grouping with M02 (AMPA) for which sufficient genotoxicity data is available. However, the RMS notes that less stringent criteria for similarity were used and therefore reliability of the read-across is questionable.

For M06 and M07 the authors propose read across to M04 and M05 for which a data gap to address aneugeniticity have been set. Moreover, as indicated above the reliability of the read-across is questionable.

Table B.6.8.1.1.11-1: **Read-across grouping**

Substances	Gene mutation	Structural chromosome aberration	Numerical chromosome aberration	Final evaluation
Group 1				
Glyphosate (P01)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
***N*-acetyl glyphosate (M04)**	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
***N*-acetyl AMPA (M05)**	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
***N*-glyceryl AMPA (M06)**	QSAR+RA(M04,M05) Negative	QSAR+RA(M04,M05) Negative	QSAR+RA(M04,M05) Negative	Non genotoxic
***N*-malonyl AMPA (M07)**	QSAR+RA(M04,M05) Negative	QSAR+RA(M04,M05) Negative	QSAR+RA(M04,M) Negative	Non genotoxic
Group 2				
Glyphosate (P01)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
AMPA (M02)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
***N*-methyl AMPA (M03)**	QSAR+RA(P01,M02)	QSAR+RA(P01,M02)	QSAR+RA(P01,M02)	Non genotoxic
Group 3				
Glyphosate (P01)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
AMPA (M02)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
Methyl phosphonic acid (M08)	QSAR+RA(P01,M02)	QSAR+RA(P01,M02)	QSAR+RA(P01,M02)	Non genotoxic
Group 4				
Glyphosate (P01)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
N-acetyl glyphosate (M04)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
N-methyl glyphosate (M09)	EXP+QSAR Negative	QSAR+RA(P01,M04)	QSAR+RA(P01,M04)	Non genotoxic

Dark green = experimental data available.
Light green = read-across

Conclusion study authors:
The grouping proposed above was developed for genotoxicity as a whole and therefore applies to chromosome (numerical and structural) aberration as well. With this strategy, chromosome aberration of metabolites M03, M06, M07, M08 and M09 can be covered with data available for the parent substance, M02, M04 and M05. Moreover, the grouping allows to disregard the structural alert identified by TOXTREE and supports the

negative predictions (no alert) obtained by DEREK. To conclude, the (Q)SAR results support non-genotoxicity of glyphosate and the metabolites for which experimental data are available (M02, AMPA; M04, *N*-acetyl glyphosate; M05, *N*-acetyl AMPA and *N*-methyl glyphosate; M09). *N*-methyl AMPA (M03), *N*-glyceryl-AMPA (M06), *N*-malonyl AMPA (M07), methyl phosphonic acid (M08) and *N*-methyl glyphosate (M09) are evaluated as non-genotoxic as well, based on the (Q)SAR and read-across evaluation ((Q)SAR and read-across genotoxicity evaluation of glyphosate and eight metabolites, Report No.: 110054/QSAR/1).

Conclusion RMS:
The RMS has some doubts about the approach used in the read-across approach, which are indicated in the summary above. Moreover, even if the proposed read-across was accepted data gaps have been set for genotoxicity for metabolites M04 and M05.

The QSAR analysis did not provide a concern for genotoxicity for glyphosate and its metabolites. However, this is not considered sufficient to fully address genotoxicity.

The applicant mentions that experimental genotoxicity data is available for N-methyl glyphosate (M09). The applicant is requested to submit the data as these were not included in the dossier.

B.6.8.1.2. Studies with N-acetyl AMPA

B.6.8.1.2.1. Acute oral toxicity

Study 1

Data point:	CA 5.8.1/032
Report author	[redacted]
Report year	2007
Report title	IN-EY252: Acute Oral Toxicity Study in Rats - Up-and-Down Procedure
Report No	[redacted]-22229
Document No	NA
Guidelines followed in study	U.S. EPA Health Effect Test Guidelines OPPTS 870.1100 (2002); OECD Guideline for the Testing of Chemicals Section 4 (Part 425) (2001)
Deviations from current test guideline (OECD 425, 2008)	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid (Category 1) **Conclusion AGG:** The study is considered to be acceptable.

Executive summary
A single dose of IN-EY252 was administered by oral gavage to 3 fasted female rats at a dose of 5000 mg/kg bw. The rats were dosed one at a time at a minimum of 48-hour intervals. The rats were observed for mortality, body weight effects and clinical signs for 14 days after dosing. All rats were necropsied to detect grossly observable evidence of organ or tissue damage.
No deaths occurred. Clinical signs of toxicity were observed in all rats up to 2 days after dosing and included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin, wet fur, ataxia and/or hyper-reactivity. No body weight losses occurred after dosing. No test substance-related gross lesions were found in the study.
Under the conditions of this study, the oral LD_{50} for IN-EY252 was greater than 5000 mg/kg bw for female rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: N-Acetyl AMPA (IN-EY252)

Identification:	[(Acetylamino)methyl]phosphonic acid
Description:	White solid
Lot/Batch #:	IN-EY252-003
Purity:	79%
Stability of test compound:	The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in colour or physical state was observed. Expiry date: 2010-05-16.

2. Vehicle and/ or positive control:	Deionised water / none
3. Test animals:	
Species:	Rat
Strain:	Crl:CD(SD)
Source:	[redacted]
Age at dosing:	10 – 11 weeks
Sex:	Female
Weight at dosing:	207.9 – 216.2 g
Acclimation period:	6 days
Diet/Food:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Individually in stainless steel, wire-mesh cages suspended above cage boards
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 30 – 70 % Air changes: Not reported 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2007-08-28 to 2007-09-12

Animal assignment and treatment:
A single oral dose of IN-EY252, suspended in deionised water, was administered by oral gavage to 3 fasted female rats at a dose of 5000 mg/kg bw. The rats were dosed one at a time at a minimum of 48-hour intervals. The rats were fasted approximately 16 – 18 hours prior to dosing, with food being returned to the rats approximately 3 – 4 hours after dosing. Individual dose volumes were calculated using the fasted body weights obtained prior to dosing. The rats were dosed at a volume of 20 mL per kg of body weight. The dosing suspensions were stirred throughout the dosing procedure.

Mortality
Viability was checked daily.

Clinical observations
Observations for signs of illness, injury or abnormal behaviour were made daily.
The rats were observed for clinical signs at the beginning of fasting, just before dosing (test day 0), once during the first 30 minutes after dosing and 2 more times on the day of dosing and once each day thereafter for the 14 day observation period.

Body weight
The rats were weighed on test days –1, 0, 7 and 14.

Sacrifice and pathology
On test day 14, the rats were anesthetised by carbon dioxide, euthanised by exsanguination and necropsied to detect grossly observable evidence of organ or tissue damage.

II. RESULTS AND DISCUSSION

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

B. CLINICAL OBSERVATIONS
Clinical signs of toxicity were observed in all rats up to 2 days after dosing and included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin (abdomen, yellow; inguen, yellow or brown; perineum, yellow or brown), wet fur (perineum), ataxia and/or hyper-reactivity.

C. BODY WEIGHT
No body weight losses occurred after dosing.

D. NECROPSY
Gross pathology
No test substance-related gross lesions were found in the study. The only gross lesion observed, uterus dilatation in rat 3308, was non-specific and not indicative of target organ toxicity.

III. CONCLUSIONS
Under the conditions of this study, the oral LD_{50} for IN-EY252 was greater than 5000 mg/kg bw for female rats.

Assessment and conclusion by applicant:
This study is considered to be valid as it was conducted under GLP and no deviations from the recent guideline could be identified. Under the conditions of this study, the oral LD_{50} for IN-EY252 was greater than 5000 mg/kg bw for female rats.

Assessment and conclusion by RMS:
It is agreed with the applicant that the acute oral LD_{50} of IN-EY252 in female rats was determined to be > 5000 mg/kg bw in this study. The study was not available for the previous evaluation (RAR, 2015).

B.6.8.1.2.2. Short-term toxicity

Study 1

Data point	CA 5.8.1/033
Report author	[redacted]
Report year	2008
Report title	IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats
Report No	[redacted]23316
Document No	[redacted]-189194
Guidelines followed in study	OPPTS 870.3100 (1998); OECD Guideline 408 (1998)
Deviations from current test guideline (OECD 408, 2018)	Clinical chemistry was performed without determining LDL, HDL, T3, T4 and TSH; organ weights were determined without prostate (+seminal vesicles and coagulating glands), thyroid and pituitary gland; histopathology was performed without coagulating glands, gall bladder and male mammary glands.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

Executive summary
In a 90-day feeding study, IN-EY252 technical was administered to male and female Crl:CD(SD) rats (10 rats/sex/concentration) at concentrations of 0, 900, 6000, and 18000 ppm (equivalent to 0, 55, 374 and 1163 mg/kg bw/day for males and 0, 68, 455 and 1400 mg/kg bw/day for females). Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, abbreviated functional observational battery (FOB), haematology, clinical chemistry, urinalysis, ophthalmology, organ weights and gross and microscopic pathology.
No adverse effects were noted in any of the parameters examined. Test substance-related clinical signs, soft faeces, and dried and/or wet brown material around the anogenital area were observed in 18000 ppm group males and females. Final body weights (test day 91) and overall body weight gains (test day 0 – 91) in males and females were 7.6% and 5.0% and 11.5% and 9.3%, respectively, lower (statistically non-significant) than controls in the 18000 ppm group. These changes were considered test substance related, but not adverse according to the applicant.

In contrast to the applicant, the RMS proposes a NOAEL of 6000 ppm based on abnormal excreta both sexes and a decreased body weight gain in males (-12%) at the LOAEL of 18000 ppm.
Therefore, the NOAEL of this study is 374 mg/kg bw/day in males and 455 mg/kg bw/day in females.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	N-Acetyl AMPA (IN-EY252)
Identification:	[(Acetylamino)methyl]phosphonic acid
Description:	Solid, off-white powder
Lot/Batch #:	(IN-EY252-) 003
Purity:	79 %
Stability of test compound:	The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in colour or physical state was observed. Expiry date: 2010-05-16.
2. Vehicle and/ or positive control:	Diet / none
3. Test animals:	
Species:	Rat
Strain:	Crl:CD(SD)
Source:	[redacted]
Age:	Approximately 8 weeks
Sex:	Male and female
Weight at dosing:	♂ 237 – 333 g; ♀ 164 – 212 g
Acclimation period:	20 days
Diet/Food:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Reverse osmosis-treated (on-site) drinking water, *ad libitum*
Housing:	Individually in stainless steel, wire-mesh cages suspended above cage boards

Environmental conditions:	Temperature:	22 ± 3 °C (21.8 – 22.1 °C)
	Humidity:	50 ± 20 % (36.7 – 62.4 %)
	Air changes:	10 air changes / hour
	12 hours light / dark cycle	

B. STUDY DESIGN AND METHODS

In life dates: 2007-08-20 to 2007-11-19/20

Animal assignment and treatment:
The test substance, IN-EY252, was administered on a continuous basis in the basal diet for 90 or 91 days to 3 groups (Groups 2 – 4) of Crl:CD(SD) rats. Dose levels were 900, 6000 and 18000 ppm. A concurrent control group (Group 1) received the basal diet on a comparable regimen. Each group consisted of 10 animals per sex.

Table 6.8.1.2.2.1-33: IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted] 2008): Study design

Test group	Dietary concentration [ppm][1]	Mean daily intake mg/kg bw/day	Number of animals	
			Males	Females
Control	0	♂: 0; ♀: 0	10	10
Low	900	♂: 55; ♀: 68	10	10
Intermediate	6000	♂: 374; ♀: 455	10	10
High	18000	♂: 1163; ♀: 1400	10	10

[1] Concentration of IN-EY252 in ppm of active ingredient using a correction factor of 1.266.

Analysis of the test substance
Prior to the initiation of dose administration, quadruplicate samples (approximately 25 g each) for homogeneity determination were collected from the top, middle and bottom strata of the 900, 6000 and 18000 ppm test diet preparations. In addition, quadruplicate samples (approximately 25 g each) for stability determinations were collected from the middle strata of these same test diet preparations at dietary levels of 900 and 18000 ppm and stored at room temperature or refrigerated for 7 or 15 days. Quadruplicate samples (approximately 25 g each) for concentration analyses were collected from the middle stratum of each test diet preparation, including the diet preparation administered to the control group, prepared on study week 0, study week 6 and study week 12. Two samples from each set of 4 samples collected were shipped frozen to the Sponsor, DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, Delaware, for analysis. The remaining samples from each sample set were kept frozen at [redacted], and were retained as back-up samples. In addition, a 100 g sample of the basal diet was shipped to the sponsor with each shipment of test diet samples. The basal diet was used by the sponsor to prepare matrix-matched analytical chemistry standards for the homogeneity, stability and confirmation of concentration analyses.

Mortality
All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity.

Clinical observations
Clinical examinations were performed once daily for all animals. The daily observations were performed at approximately the same time each day and were omitted on days of detailed physical examinations. All significant findings were recorded. Detailed physical examinations were conducted on all animals weekly, beginning 1 week prior to test substance administration and prior to the scheduled necropsy. A separate computer protocol was used to record any observations noted outside of the above-specified intervals.

Body weight
Individual body weights were recorded at least weekly, beginning 3 weeks prior to test substance administration (study week -3). Mean body weights and mean body weight changes were calculated for the corresponding intervals. Final body weights were recorded on the day prior to (un-fasted) and on the day of (fasted) the scheduled necropsy.

Food consumption and test substance intake
Individual food consumption was recorded weekly, beginning 3 weeks prior to test substance administration (study week -3 to -2). Food intake was calculated as g/animal/day for the corresponding body weight intervals.

Food efficiency (body weight gained as a percentage of food consumed) was also calculated and reported for these intervals.
The mean amounts of test substance consumed (mg/kg bw/day) by each sex of each dietary group was calculated from the mean food consumed (g/kg of bw/day) and the appropriate target concentration of test substance in the food (mg/kg of diet).

Abbreviated Functional Observational Battery (FOB)
Abbreviated functional observational battery (FOB) assessments were recorded for all animals prior to the initiation of dose administration and during study week 12. Testing was performed by the same trained technicians, whenever possible, without knowledge of the animals' group assignments. The FOB was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70 ± 10 dB, to minimise environmental variations in the test conditions. The FOB used at [redacted] is based on previously developed protocols (Moser et al., 1991; Irwin, 1968; Gad, 1982; Moser et al., 1988; Haggerty, 1989; O'Donoghue, 1989). All animals were observed for the following parameters as described below:

1. Home Cage Observations: Faeces consistency
2. Sensory Observations: Approach response, startle response, pupil response, touch response and tail pinch response
3. Neuromuscular Observations: Grip strength - hind and forelimb

Locomotor activity
Locomotor activity was assessed for all animals prior to the initiation of dose administration and during study week 12. Locomotor activity, recorded after the completion of the abbreviated FOB, was measured automatically using the SDI Photobeam Activity System (San Diego Instruments, Inc., San Diego, California). This personal computer-controlled system utilised a series of infrared photobeams surrounding a clear plastic, rectangular cage to quantify the motor activity of each animal. Four-sided black plastic enclosures were used to surround the clear plastic boxes and decrease the potential for distraction from extraneous environmental stimuli or activity by technicians or adjacent animals. The black enclosures rested on top of the photobeam frame and did not interfere with the path of the beams. The locomotor activity assessment was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70 ± 10 dB, to minimise environmental variations in the test conditions. The testing of treatment groups was conducted according to replicate sequence. Each animal was tested separately. Data were collected in 5-minute epochs and the test session duration was 60 minutes. These data were compiled as four 15-minute sub-sessions for tabulation.
Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills (i.e., grooming, interruption of 1 photobeam) and ambulatory motor activity (interruption of 2 or more consecutive photobeams).

Ophthalmoscopic examination
Ocular examinations were conducted on all animals prior to the initiation of dose administration (study week -3) and near the end of the treatment period (study week 12). All ocular examinations were conducted using an indirect ophthalmoscope and slit lamp biomicroscope preceded by pupillary dilation with tropicamide ophthalmic solution, USP, 0.5 %.

Haematology, clinical chemistry and urinalysis
Blood and urine samples for clinical pathology evaluations (haematology, coagulation, serum chemistry and urinalysis) were collected from all surviving animals at the scheduled necropsy (study week 13). In addition, blood samples for clinical pathology evaluations (haematology, coagulation and serum chemistry) were collected and analysed from the Group 3 male euthanised *in extremis* (prior to euthanisation). The animals (excluding the animal euthanised *in extremis*) were fasted overnight prior to blood collection while in metabolism cages for urine collection. Blood was collected for haematology and serum chemistry evaluation via the retro-orbital sinus of animals anesthetised by inhalation of isoflurane. Blood samples for analysis of coagulation parameters were collected at the time of euthanasia via the *vena cava* of animals' euthanised by inhalation of carbon dioxide. Blood was collected into tubes containing EDTA (haematology), sodium citrate (clotting determinations) or no anticoagulant (serum chemistry).

The following haematological parameters were evaluated: Blood smears, total leukocyte count, erythrocyte count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, prothrombin time, activated partial

thromboplastin time (APTT), reticulocyte count (percent/absolute) and differential leukocyte count (percent and absolute: Neutrophil, lymphocyte, monocyte, eosinophil, basophil and large unstained cells).

The following clinical chemistry parameters were evaluated: Albumin, total protein, globulin, albumin/globulin ratio (A/G Ratio), total bilirubin (Total Bili), urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl-transferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, sodium, sorbitol dehydrogenase and triglycerides.

The following urinalysis parameter were evaluated: Specific gravity, pH, urobilinogen, total volume, colour, clarity, protein, glucose, ketones, bilirubin, occult blood, leukocytes, nitrites, microscopy of sediment and osmolality.

Sacrifice and pathology
Gross pathology
A complete necropsy was conducted on all animals. Animals were euthanised by carbon dioxide anaesthesia and exsanguinated. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities, including viscera.

Organ weights
The following organs were weighed from all animals at the scheduled necropsy: Adrenals, brain, epididymides, heart, kidneys, liver, ovaries with oviducts, spleen, testes, thymus and uterus. Paired organs were weighed together. Organ / body weight and organ / brain weight ratios were calculated.

Histopathology
After fixation, protocol-specified tissues were trimmed according to standard operating procedures and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 microns, mounted on glass microscope slides and stained with haematoxylin and eosin.
The following organs were examined: Adrenals, aorta, bone with marrow (with femur and sternum), bone marrow smear, brain, cervix, epididymides, exorbital lacrimal glands, eyes with optic nerve, oesophagus, stomach, duodenum, jejunum, ileum, Peyer's Patches, caecum, colon, rectum, heart, kidneys, larynx, liver, lungs, lymph nodes (mandibular and mesenteric), nasal cavity, mammary gland (females only), ovaries (with oviducts), pancreas, peripheral nerve (sciatic), pharynx, pituitary, prostate, salivary glands (mandibular), seminal vesicles, skeletal muscle (rectus femoris), skin, spinal cord (cervical, thoracic, lumbar), spleen, testes, thymus, thyroid/parathyroids, tongue, trachea, urinary bladder, uterus, vagina and all gross lesions and masses.

Statistics
Body weight, food intake parameters, abbreviated FOB, clinical pathology, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used. Functional observational battery parameters that yielded scalar or descriptive data were analysed using Fisher's Exact Test. Non-parametric statistical analysis was conducted for gamma glutamyl transferase.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSIS
Homogeneity and stability were verified prior to study start and concentration verification was done 3 times during the study (start, middle and end). The test substance was at target concentrations (95.6 – 109.4 % nominal), homogeneous (94.2 – 102.2 % nominal) throughout the feed, and was stable (88.3 – 111.1 %) for up to 15 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

B. MORTALITY
One 6000 ppm group male was euthanised *in extremis* on study day 36, which was associated with nasal cavity fractures and was unrelated to test substance administration. Clinical observations noted in this male included dermal atonia, gasping, upper and lower incisors malaligned/broken, red material around the left and right eyes, soft faeces, decreased defecation, smaller than normal faeces, dried yellow material around the urogenital area and dried brown material around the anogenital area.

Additionally one control group male was found dead on study day 71. The cause of death was undetermined, as there were no significant or remarkable clinical findings for this animal throughout the study or at the unscheduled necropsy.

None of the deaths was related to treatment.

C. CLINICAL OBSERVATIONS

Clinical findings were primarily limited to the 18000 ppm group males and females and included occurrences of soft faeces and dried and/or wet brown material around the anogenital area. These clinical findings were considered to be test substance-related, but non-adverse by the applicant. The RMS, however, considers these effects as adverse. Other clinical signs were common findings for laboratory rats of this age and strain.

D. BODY WEIGHT

There were no adverse effects on body weights or body weight gains. Final body weights (test day 91) were 7.6 % and 5 % lower than controls in the 18000 ppm males and females, respectively. Overall (test day 0 – 91) body weight gains were 11.5 % and 9.3 % lower than controls in the 18000 ppm males and females, respectively (or there were no test substance-related effects on body weights or body weight gains). These changes were not statistically significant and not considered adverse by the applicant. No changes were observed in lower groups. The RMS, however, considers the decreased body weight gain (-12% compared with controls) in males at 18000 ppm as adverse and treatment-related.

Table 6.8.1.2.1.1-34: IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2008): Body weight, body weight gain, food consumption and food efficiency

	Dose group [ppm]							
	Males				**Females**			
	0	**900**	**6000**	**18000**	**0**	**900**	**6000**	**18000**
Final body weight [g], (% control)	593	↓561 (95 %)	↓563 (95 %)	↓548 (92 %)	305	↑312 (102 %)	↓302 (99 %)	↓290 (95 %)
Body weight gain [g], (% control)	304	↓277 (91 %)	↓282 (93 %)	↓269 (88 %)	118	↑123 (104 %)	↓115 (97 %)	↓107 (91 %)

E. FOOD COMSUMPTION; FOOD EFFICIENCY AND DAILY INTAKE

There were no test substance-related effects on food consumption and food efficiency. The mean daily intakes for male rats were 0, 55, 374 and 1163 mg/kg bw/day. The mean daily intakes for female rats were 0, 68, 455 and 1400 mg/kg bw/day.

F. OPHTHALMOLOGICAL EXAMINATIONS

There were no test substance-related ophthalmoscopically visible changes in the eyes of either male or female rats at any dietary concentration.

G. NEUROBEHAVIORAL EVALUATIONS

There were no definitive test substance-related effects on sensory or neuromuscular parameters at the study week 12 abbreviated FOB evaluations.

Home cage observations

Test substance-related effects on home cage parameters included a statistically significantly greater number of 18000 ppm group males (10/10 animals) and females (7/10 animals) with partially formed pellets (faeces consistency) in comparison to the control group males (0/10 animals) and females (0/10 animals) during the study week 12 evaluation. These home cage observations are consistent with the abnormal excreta observations (soft faeces and dried and/or wet brown material around the anogenital area) noted in the 18000 ppm group animals during the daily clinical examinations and are considered to be non-adverse.

Sensory Observations

There were no test substance-related effects on sensory parameters when the test substance-treated males and females were compared to the control group at the study week 12 abbreviated FOB evaluations.

Neuromuscular Observations

There were no test substance-related effects on neuromuscular parameters when the test substance-treated males and females were compared to the control group at the study week 12 abbreviated FOB evaluations. Males in the 18000 ppm group had slightly lower forelimb and hind limb grip strength compared to the control group during the study week 12 evaluation. Since the difference was also present at the pre-test evaluation, the slightly lower grip strength during the study week 12 evaluation in the 18000 ppm group males was not considered to be test substance-related. Females in the 18000 ppm group also had slightly lower grip strength compared to the control group during the study week 12 evaluation. Since cumulative body weight gain for the 18000 ppm group males and females was slightly lower compared to the respective control group, the slightly lower grip strength in the 18000 ppm group males and females may also be secondary to the lower cumulative body weight gain in this group

Locomotor Activity
Within-session repeated measures analyses of variance were conducted across the subintervals of each test session for total and ambulatory counts and for overall interval means (representing the entire 60-minute session activity) during each test session. Locomotor activity patterns (mean ambulatory and total motor activity counts) were unaffected by test substance administration. There were no statistically significant differences for the test substance-treated males and females when compared to the control group during study week 12. Values obtained from the four epochs (0 – 15 minutes, 16 – 30 minutes, 31 – 45 minutes and 46 – 60 minutes) evaluated and the overall 60-minute test session values were comparable to the concurrent control values. No remarkable shifts in the pattern of habituation occurred in any of the test substance-treated groups when the animals were evaluated during study week 12.

H. HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALIYIS

There were no test substance-related alterations in haematology, serum chemistry and urinalysis parameters.

Haematology
A statistically significant higher prothrombin time in the 18000 ppm group males was noted at study week 13 but was not toxicologically significant because the increase was very small).

Table 6.8.1.2.2.1-35: IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2008): Selected haematological parameters

Parameter	Dose group [ppm]							
	Males				Females			
	0	900	6000	18000	0	900	6000	18000
Prothrombin time [s]	13.1 ± 0.56	↑13.4 ± 0.43 (102%)	↑13.5 ± 0.81 (103%)	**↑14.3* ± 1.26 (109%)**	12.6 ± 0.36	↓12.3 ± 0.58 (102%)	↓12.3 ± 0.60 (102%)	↓12.4 ± 0.53 (102%)

*: Statistically significant from control ($p<0.05$)

Clinical Chemistry
There were no test substance-related alterations in serum chemistry parameters.

Urinalysis
Urine pH was 9 % lower in the 18000 ppm group males at study week 13. Eighty percent of the 18000 ppm group males had a urine pH of 6.0, compared with 0 %, 10 % and 20 % of 0, 900 and 6000 ppm group males at study week 13. Urine volume was 51 % lower and osmolality was 41 % higher in the 18000 ppm group males at study week 13 and the small change in urine pH may have been related to concentrated urine and was not adverse.

Table 6.8.1.2.2.1-36: IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2008): Selected urinalysis parameters

Parameter	Dose group [ppm]							
	Males				Females			
	0	900	6000	18000	0	900	6000	18000
pH value	6.7 ± 0.26	↓6.5 ± 0.44	↓6.6 ± 0.39	**↓6.1** ± 0.21**	6.3 ± 0.35	↑6.5 ± 0.41	↓6.1 ± 0.46	↓6.2 ± 0.35
Urine volume [mL]	8.9 ± 4.51	↓6.7 ± 3.92	↓8.7 ± 5.24	↓4.4 ± 1.96	8.7 ± 10.52	↓5.5 ± 4.3	↓4.1 ± 1.73	↓5.6 ± 2.95
Osmolality [mOsm/kg]	1378 ±	↑1388 ±	↓1275 ±	↑1944 ±	1089 ±	↑1215 ±	↑1433 ±	↓1012 ±

	418.6	521.4	531.3	618.1	484.7	568.9	615.4	453.3

**: Statistically significant from control ($p<0.01$)

I. NECROPSY

No test substance-related changes in mean organ weights (absolute or relative) and gross lesions were observed at any dietary concentration. There were no test substance-related microscopic findings in the male or female 18000 ppm groups.

Histopathology

Inflammation was multifocally present within the lungs of individuals throughout the control and 18000 ppm groups (6/10 and 8/10 males and 5/10 and 8/10 females in the control and 18000 ppm groups, respectively). A mixed population of inflammatory cells consisting primarily of mononuclear cells (macrophages and lymphocytes) occasionally admixed with low numbers of neutrophils was typically noted in perivascular areas. Occasionally, inflammatory infiltrate extended into adjacent parenchyma and was associated with septal thickening and alveolar infiltration. This idiopathic pulmonary lesion has been described in multiple rat strains, and the most severe lesions occur in 10 – 12 week old animals. Lesions appear to be transient, with apparent resolution in older rats, and etiology remains undetermined although a viral agent is suspected (Elwell *et al*., 1997; Riley *et al*., 1997; Slaoui *et al*., 1998). The inflammatory lesion observed in this study is consistent with those reported and the presence of this lesion did not hinder histopathological evaluation of potential test substance-related effects. There were no test substance-related alterations in the lungs.

Remaining histologic changes were considered to be incidental findings or related to some aspect of experimental manipulation other than administration of the test substance. There was no test substance-related alteration in the prevalence, severity or histologic character of those incidental tissue alterations.

III. CONCLUSIONS

There were no indications of systemic toxicity when IN-EY252 was administered on a continuous basis in the basal diet for 90 or 91 days to 3 groups (Groups 2 – 4) of Crl:CD(SD) rats at dose levels of 900, 6000 and 18000 ppm. IN-EY252 was well tolerated with no adverse effects on clinical observations, body weights, food consumption, FOB parameters and clinical pathology parameters. In addition, there were no ophthalmic, histologic or gross alterations related to test substance administration. Therefore, the no-observed-adverse-effect level (NOAEL) in this study for administration of IN-EY252 on a continuous basis in the basal diet for 90 or 91 days to Crl:CD(SD) rats was 18000 ppm (equivalent to 1163 and 1400 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by applicant:

Despite the deviations in clinical chemistry (LDL, HDL, T3, T4 and TSH not determined), organ weights (prostate + seminal vesicles and coagulating glands), thyroid and pituitary gland not determined) and histopathology (coagulating glands, gall bladder and male mammary glands not examined) this study was conducted under GLP and was considered to be valid.

Based on a lack of adverse effects on in-life parameters, neurobehavioral evaluation and clinical and anatomic pathology in males and females at any dose level, the NOAEL for males and females was 18000 ppm (equivalent to 1163 and 1400 mg/kg bw/day, respectively).

Assessment and conclusion by RMS:

In contrast to the applicant, the RMS proposes a NOAEL of 6000 ppm based on abnormal excreta both sexes and a decreased body weight gain in males (-12%) at the LOAEL of 18000 ppm.

Therefore, the NOAEL of this study is 374 mg/kg bw/day in males and 455 mg/kg bw/day in females.

B.6.8.1.2.3. Genotoxicity

Study 1

Data point	CA 5.8.1/034
Report author	[redacted]
Report year	2007
Report title	IN-EY252: Bacterial Reverse Mutation Assay

Report No	DuPont-22227
Document No	AB47BT.503.BTL
Guidelines followed in study	OECD 471 (1998), EEC Commission Directive 2000/32/EC Annex 4D-B.13/14 (2000), US EPA OPPTS 870.5100, JMAFF 12 NouSan 8147 (2000 and 2001)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 1) **Conclusion AGG:** The study is considered to be acceptable.

Executive summary
[(Acetylamino)methyl]phosphonic acid, metabolite of glyphosate, was investigated for its potential to cause gene mutation in bacteria (Ames test). *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were exposed to the test item, solvent (water) and appropriate positive controls in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). A preliminary cytotoxicity and mutagenicity experiment was performed to identify cytotoxic concentrations of the test item. In a standard plate test (plate incorporation method), test item concentrations in the range of 1.5 to 5000 µg/plate were applied in the presence and absence of S9 mix. No cytotoxicity was observed in any tester strain up to the highest tested concentration, neither in the presence, nor in the absence of S9 mix.
The preliminary experiment was designated Experiment B1 of the main mutation assay. However, as no bacterial growth was observed on the tester strain plates TA 1535, TA 1537 and WP2 uvrA in the presence and absence of S9 mix, a repeat experiment was conducted with the respective strains (Experiment B2). In addition, a second standard plate test (Experiment B3) was performed using concentrations in the range of 50 – 5000 µg/plate. Both experiments were performed with triplicate plates. After 48 – 72 hours of incubation at 37 ± 2 °C, the bacterial background lawn was examined and the number of revertant colonies were counted for each plate.
Precipitation of the test item in vehicle or cytotoxicity, evident as a reduction in the number of revertant colonies or in the bacterial background lawn were not observed for any strain in any tested concentration, neither with nor without S9 mix.
There was no statistically significant and biologically relevant increase in the number of his^+ or trp^+ revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of metabolic activation. The number of revertant colonies induced by the solvent and the positive controls were within the range of the laboratories historical control data, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.
Under the conditions of the test, [(Acetylamino)methyl]phosphonic acid, metabolite of glyphosate, is not mutagenic in the Ames standard plate test with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: [(Acetylamino)methyl]phosphonic acid (metabolite of glyphosate)

Identification:	IN-EY252
Description:	White powder
Lot/Batch number:	IN-EY252-001
Purity:	76%
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark with desiccant) was guaranteed until the reported expiry date 25 Apr 2009. The stability of the test item in solvent was confirmed by analytical methods.
Solvent (vehicle) used:	Water

2. Control materials:

Negative control:	A negative control was not employed in this study.
Solvent (vehicle) control:	Water

Solvent (vehicle)/final concentration:	0.1 mL per plate.
Positive controls:	Please refer to table below.

Strain	**Metabolic activation**	**Mutagen**	**Conc. [µg/plate]**
S. typhimurium strains			
TA 100	-S9	Sodium azide	1.0
	+S9	2-Aminoanthracene[#]	1.0
TA 1535	-S9	Sodium azide	1.0
	+S9	2-Aminoanthracene[#]	1.0
TA 98	-S9	2-Nitrofluorene	1.0
	+S9	2-Aminoanthracene[#]	1.0
TA 1537	-S9	9-Aminoacridine	75.0
	+S9	2-Aminoanthracene[#]	1.0
E. coli strains			
WP2 uvrA	-S9	Methyl methanesulfonate	1000.0
	+S9	2-Aminoanthracene[#]	10.0

[#]: The functionality of the S9 mix batch used was additionally checked with 7,12-dimethylbenz(a)anthracene and showed the expected results.

3. Metabolic activation:

S9 mix was purchased from [redacted] and obtained from the livers of male Sprague-Dawley rats. The animals received a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bw. The livers were prepared 5 days after treatment. The S9 mix was thawed prior to each experiment and co-factors were immediately added to prepare S9 mix containing the following components:

S9 mix component	**Concentration**	**Unit**
Phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				**Bacteria batch checked for**	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

1. **Preliminary cytotoxicity and mutagenicity test / Experiments B1 & B2 of the main mutation assay:**

Plate incorporation test ± S9 mix:	
Concentrations:	1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate[*]
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2uvrA
Replicates:	Duplicate

[*]*: Dosing solutions were adjusted to compensate the purity of the test substance (76 %) by using a correction factor of 1.45.*

2. **Experiment B3 of the main mutation assay:**

Plate incorporation test ± S9 mix:	
Concentrations:	50, 150, 500, 1500 and 5000 µg/plate*
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2uvrA
Replicates:	Triplicates

*: Dosing solutions were adjusted to compensate the purity of the test substance (76 %) by using a correction factor of 1.45.

B: Study design and methods
Dates of experimental work: 01 – 21 Mar 2007
Finalisation date: 23 Jul 2007

1. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution, vehicle or positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation only) or S9 substitution buffer (in tests without S9 mix) were added to 2 mL of molten selective top agar (supplemented with 0.05 mM L-histidine + 0.05 mM L-biotin or 0.05 mM L-tryptophan). When plating the positive controls, the test item solution was replaced by 50 µL aliquot of the respective positive control. After vortexing, the mixture was overlaid onto the surface of a minimal bottom agar plate. After the overlay had solidified, the plates were inverted and incubated for approx. 48 – 72 hours at 37 ± 2 °C. Following incubation, the bacterial background lawn was examined and the number of his^+ revertant colonies were counted. Plates that were not counted immediately following the incubation period were stored at 2 – 8 °C until evaluation. Each concentration and the controls were tested in duplicates (experiment B1 and B2) or triplicates (experiment B3).

2. Cytotoxicity
Toxicity was detected by a

- reduction in the number of spontaneous revertants by > 50 %. The reduction must have been accompanied by an abrupt dose-dependent drop in the revertant count.
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

3. Statistics
For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated. No further statistical analysis was performed.

4. Acceptance criteria
The test was considered valid if the following criteria were met:

- All bacterial cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls.
- To ensure that appropriate numbers of bacteria were plated, tester strain cultures must have been greater or equal to 0.3×10^9 cells/mL.
- The mean of each positive control exhibited at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.
- A minimum of 3 non-toxic dose levels were included for the evaluation of data.

5. Evaluation criteria
A test item was considered positive (mutagenic) in the assay if the following criteria were met:

- There was a dose-related increase in the mean number of revertants per plate in at least one tester strain over a minimum of two increasing concentrations of the test substance when compared to the vehicle control.
- The increase in the mean number of revertant colonies per plate were at least 3.0-fold for tester strains TA 1535 and TA 1537 and at least 2.0-fold for tester strains TA 98, TA 100 and WP2uvrA.

An equivocal response was obtained if there was a biologically relevant increase in the number of revertants that partially met the criteria for evaluation as a positive substance. This was for example the case if a dose-responsive increase did not achieve the respective threshold or if a non-dose-responsive increase was equal to or greater than the respective threshold.
A test item was considered negative (non-mutagenic) if it was neither evaluated positive nor equivocal.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS

Analytical determinations of test item solutions in vehicle were performed for the concentrations 0.5, 5 and 50 mg/mL. Concentrations were measured by high performance liquid chromatography (HPLC) and UV detection. The actual concentrations were between 106 – 110 % of the target, meeting the acceptance criteria of 85 – 115 % of target. There was no test substance in the 0 mg/mL sample. Stability analysis showed that the test substance in vehicle was stable for approx. 4 hours when stored at room temperature.

2. CYTOTOXICITY

In the preliminary toxicity and mutagenicity test (Experiments B1 and B2) of the main mutation assay there was no cytotoxicity observed up to the highest tested concentration, neither in the presence, nor absence of metabolic activation. Due to the lack of bacterial growth on all assay plates, tester strains TA 1535, TA 1537 and WP2 uvrA in the presence and absence of S9 mix were not evaluated in experiment B1, but were re-tested in an independent experiment (Experiment B2).
In the second experiment of the main mutagenicity assay (Experiment B3) there was no cytotoxicity in any tester strain at any tested concentration, neither in the presence nor absence of S9 mix.

3. SOLUBILITY

Precipitation of the test substance in vehicle was not observed.

4. MUTATION ASSAY

There was no biologically relevant increase in the number of his^+ or trp^+ revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of metabolic activation.
The number of revertant colonies induced by the solvent and the positive controls were within the range of the laboratories historical control data, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.

Table B.6.8.1.2.3.1-1: [(Acetylamino)methyl]phosphonic acid, metabolite of glyphosate (IN-EY252) - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 2007), preliminary cytotoxicity and mutagenicity assay (Experiment B1 and B2)

Preliminary cytotoxicity and mutagenicity test (Experiment B1 and B2): Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535§**		**TA 1537§**		**WP2 uvrA§**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	19	26	114	85	17	11	4	7	22	17
± SD	*± 4*	*± 1*	*± 24*	*± 2*	*± 4*	*± 1*	*± 1*	*± 2*	*± 10*	*± 1*
HCD# mean	18	26	146	156	18	15	7	7	17	18
± SD	*± 6*	*± 9*	*± 32*	*± 34*	*± 7*	*± 5*	*± 3*	*± 3*	*± 6*	*± 7*
[range]	*5 – 51*	*8 – 72*	*63 – 253*	*67 – 267*	*4 – 49*	*2 – 45*	*1 – 23*	*1 – 25*	*5 – 58*	*6 – 52*
Test item [µg/plate]										
1.5 mean	16	26	137	112	18	9	6	7	18	19
± SD	*± 1*	*± 1*	*± 4*	*± 2*	*± 1*	*± 4*	*± 2*	*± 2*	*± 1*	*± 1*
5.0 mean	17	17	126	120	22	13	7	7	19	24
± SD	*± 4*	*± 1*	*± 4*	*± 11*	*± 0*	*± 1*	*± 3*	*± 3*	*± 3*	*± 4*
15 mean	14	18	100	114	18	13	4	8	20	20
± SD	*± 5*	*± 1*	*± 8*	*± 43*	*± 1*	*± 3*	*± 0*	*± 1*	*± 2*	*± 1*
50 mean	18	23	123	124	19	15	5	6	14	20
± SD	*± 5*	*± 4*	*± 3*	*± 9*	*± 3*	*± 6*	*± 1*	*± 1*	*± 8*	*± 1*
150 mean	14	27	147	128	15	15	3	6	19	22

Preliminary cytotoxicity and mutagenicity test (Experiment B1 and B2): Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535[§]**		**TA 1537[§]**		**WP2 uvrA[§]**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
± SD	*± 1*	*± 1*	*± 4*	*± 10*	*± 0*	*± 4*	*± 2*	*± 1*	*± 2*	*± 6*
500 mean	15	25	123	101	18	8	4	6	18	17
± SD	*± 3*	*± 1*	*± 7*	*± 8*	*± 0*	*± 1*	*± 2*	*± 1*	*± 2*	*± 0*
1500 mean	16	27	137	122	14	13	5	8	25	28
± SD	*± 1*	*± 4*	*± 1*	*± 11*	*± 0*	*± 4*	*± 2*	*± 0*	*± 8*	*± 3*
5000 mean	17	28	122	115	14	14	7	11	16	17
± SD	*± 5*	*± 1*	*± 8*	*± 11*	*± 3*	*± 2*	*± 4*	*± 1*	*± 1*	*± 2*
Positive control										
mean	221	416	467	492	325	95	326	65	263	133
± SD	*± 24*	*± 34*	*± 57*	*± 62*	*± 54*	*± 5*	*± 127*	*± 9*	*± 7*	*± 19*
HCD[#] mean	212	781	586	959	370	139	690	120	151	520
± SD	*± 175*	*± 434*	*± 136*	*± 427*	*± 137*	*± 70*	*± 373*	*± 113*	*± 110*	*± 271*
[range]	*44 - 1981*	*42 - 2669*	*239 - 2373*	*168 - 2652*	*31 - 1050*	*21 - 985*	*14 - 2216*	*13 - 2021*	*32 - 1741*	*35 - 1392*

[§] = data were obtained in a separate experiment (Experiment B2) because no bacterial growth was observed for these strains in the first experiment (Experiment B1, data not shown).

[#] = Historical control data generated from 2003 - 2005

Table B.6.8.1.2.3.1-2: [(Acetylamino)methyl]phosphonic acid, metabolite of glyphosate (IN-EY252) - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 2007), Experiment B3

Experiment B3: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	15	18	118	146	20	14	6	6	20	24
± SD	*± 3*	*± 1*	*± 15*	*± 20*	*± 1*	*± 2*	*± 3*	*± 2*	*± 1*	*± 5*
HCD[#] mean	18	26	146	156	18	15	7	7	17	18
± SD	*± 6*	*± 9*	*± 32*	*± 34*	*± 7*	*± 5*	*± 3*	*± 3*	*± 6*	*± 7*
[range]	*5 - 51*	*8 - 72*	*63 - 253*	*67 - 267*	*4 - 49*	*2 - 45*	*1 - 23*	*1 - 25*	*5 - 58*	*6 - 52*
Test item [µg/plate]										
50 mean	15	11	126	143	19	16	7	7	31	18
± SD	*± 2*	*± 2*	*± 33*	*± 25*	*± 2*	*± 3*	*± 2*	*± 1*	*± 3*	*± 2*
150 mean	14	18	170	145	20	13	6	9	25	21
± SD	*± 5*	*± 4*	*± 39*	*± 33*	*± 3*	*± 3*	*± 3*	*± 2*	*± 8*	*± 4*
500 mean	14	12	150	110	16	13	5	8	26	27
± SD	*± 2*	*± 4*	*± 21*	*± 20*	*± 3*	*± 4*	*± 2*	*± 3*	*± 5*	*± 5*
1500 mean	16	18	114	117	17	14	9	10	22	25
± SD	*± 1*	*± 8*	*± 9*	*± 24*	*± 1*	*± 3*	*± 3*	*± 3*	*± 7*	*± 5*
5000 mean	15	18	117	150	16	13	7	8	23	18
± SD	*± 3*	*± 4*	*± 25*	*± 19*	*± 4*	*± 2*	*± 5*	*± 1*	*± 4*	*± 1*

Experiment B3: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Positive control										
[§] mean	162	290	389	438	348	90	331	60	188	190
± SD	*± 31*	*± 39*	*± 60*	*± 49*	*± 90*	*± 3*	*± 66*	*± 17*	*± 40*	*± 22*
HCD[#] mean	212	781	586	959	370	139	690	120	151	520
± SD	*± 175*	*± 434*	*± 136*	*± 427*	*± 137*	*± 70*	*± 373*	*± 113*	*± 110*	*± 271*
[range]	*44 - 1981*	*42 - 2669*	*239 - 2373*	*168 - 2625*	*31 - 1050*	*21 - 985*	*14 - 2216*	*13 - 2021*	*32 - 1741*	*35 - 1392*

[§] = information on respective positive control is reported in Material and Method section I.A.2

[#] = Historical control data generated from 2003 - 2005

III. CONCLUSIONS

Based on the experimental findings, [(Acetylamino)methyl]phosphonic acid (IN-EY252), metabolite of glyphosate, was negative for gene mutation in bacteria in the Ames standard plate test in the presence and absence of metabolic activation.

Assessment and conclusion by applicant:

In the present study, [(Acetylamino)methyl]phosphonic acid was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation.

The study was conducted under GLP conditions and in accordance with OECD guideline 471 (1997). There were no deviations from the guideline. The study is therefore considered valid.

Assessment and conclusion by RMS:

It is agreed with the applicant that in this test, [(Acetylamino)methyl]phosphonic acid was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation. The study was not available for the previous evaluation (RAR, 21015)

Study 2

Data point	CA 5.8.1/035
Report author	[redacted]
Report year	2007
Report title	IN-EY252*: In vitro* Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes
Report No	DuPont-22225
Document No	AB47BT.341.BTL
Guidelines followed in study	OECD 473 (1997), US EPA OPPTS 870.5375 (1998), EC Commission Directive 2000/32/EC Annex 4A-B10 (2000), JMAFF 12-NouSan-8147 (2000 and 2001)
Deviations from current test guideline	Only 200 cells in metaphase were evaluated (as required by the OECD guideline 473 (1997)), whereas the currently valid OECD 473 (2016) recommends the evaluation of 300 metaphase cells per condition.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 1) **Conclusion AGG:** The study is considered to be acceptable but with restrictions (reliable with restrictions) due to the deviation noted above.

Executive summary
[(Acetylamino)methyl]phosphonic acid was tested in a mammalian chromosome aberration test in peripheral human lymphocytes *in vitro* for its potential to induce structural and numerical chromosome aberrations in the presence and absence of metabolic activation (rat liver S9 fraction).
Concentrations for the cytogenicity study were selected based on the results of a preliminary toxicity test, in which no cytotoxicity was observed up to 1530 µg/mL (corresponding to 10 mM) in the presence and absence of S9 mix. Duplicate cultures were exposed to the test item, solvent (water) or positive control (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) in the presence and absence of metabolic activation. Test item concentrations ranged from 191.25 to 1530 µg/mL.
The cells were exposed for 4 hours in the presence and absence of S9 mix and for 20 hours in the absence of S9 mix. Cells of the 4-hour exposure period were washed with calcium and magnesium-free phosphate buffered saline following treatment and re-incubated in culture medium for the remaining 16 hours of incubation. All cultures were sampled 20 hours after start of exposure and chromosome preparations were made.
A total of 200 metaphase cells per condition were scored for structural and numerical chromosome aberrations. Cytotoxicity was evaluated as mitotic index and assessed for 500 cells per culture.
Precipitation of the test substance in vehicle or culture medium, or cytotoxicity, evident as a ≥ 50 % reduction in the mitotic index were not observed for any treatment schedule or any concentration, neither in the presence, nor in the absence of S9 mix.
There was no statistically significant increase in the number of cells with structural or numerical chromosomal aberrations observed for any treatment schedule, neither in the presence nor in the absence of metabolic activation. The percentage of aberrant cells in the test substance-treated groups was comparable to those of solvent controls at any dose level.
The positive and vehicle controls fulfilled the criteria for a valid test and demonstrated the functionality of the metabolic activation system and the validity of the test.
Under the conditions of the test, [(Acetylamino)methyl]phosphonic acid, metabolite of glyphosate, did not induce chromosomal aberrations in human peripheral lymphocytes *in vitro*, neither in the presence, nor in the absence of metabolic activation.

Materials and methods

A. MATERIALS

1. Test material:	[(Acetylamino)methyl]phosphonic acid (metabolite of glyphosate)
Identification:	IN-EY252
Description:	White powder
Lot/Batch number:	IN-EY252-001
Purity:	76%
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark with dessicant) was guaranteed until the expiry date 25 Apr 2009. The stability of the test item in solvent was confirmed by analytical methods.
Solvent (vehicle) used:	Water
2. Control material:	
Negative control:	The negative control was actually the solvent control.
Solvent (vehicle) control:	Water
Positive control:	- S9 mix: Mitomycin C (MMC), 0.6 µg/mL for the 4-hour treatment and 0.3 µg/mL for the 20-hour treatment + S9 mix: Cyclophosphamide (CP), 20 µg/mL

3. Metabolic activation:

S9 mix was purchased from [redacted] (Lot no. 2074) and each batch was assayed for sterility and its ability to metabolise 2-aminoanthracene and 7,12-dimethyl-benzanthracene to mutagens in *Salmonella typhimurium* strain TA 100.
Immediately prior to use, S9 was thawed and mixed with co-factors as follows:

S9 mix component	Concentration	Unit

KCl	6	mM
NADPH-generating system		
Glucose 6-phosphate	1	mM
NADP	1	mM
$MgCl_2$	2	mM
S9	2	% (v/v)

4. Test organism:

Human peripheral blood lymphocytes were obtained from a healthy, non-smoking, 25-year old female (preliminary cytotoxicity test) and from a healthy, non-smoking 30-year old female (main cytogenicity test) and collected in heparinised vessels.

5. Cell culture media:

Medium:	RPMI 1640 medium, supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin and 2 mM L-glutamine
Incubation:	Cultures were incubated at 37 ± 1 °C in a humidified atmosphere of 5 ± 1 % CO_2.
Cell culture establishment prior to exposure:	0.6 mL of heparinised blood was cultured with 9.4 mL culture medium and 0.1 % phytohaemagglutinin for 44 - 48 hours prior to treatment.

6. Test concentrations and number of replicates:

1. Preliminary cytotoxicity assay

Metabolic activation	Duration of exposure (Fixation time)	Concentrations[§]	Replicates
- S9 mix	4 h (20 h)	0.153, 0.459, 1.53, 4.59, 15.3, 45.9, 153, 459 and 1530[#] µg/mL	Single culture
- S9 mix	20 h (20 h)	0.153, 0.459, 1.53, 4.59, 15.3, 45.9, 153, 459 and 1530[#] µg/mL	Single culture
+ S9 mix	4 h (20 h)	0.153, 0.459, 1.53, 4.59, 15.3, 45.9, 153, 459 and 1530[#] µg/mL	Single culture

[#]**:** highest concentration, corresponding to 10 mM

[§]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.45.

2. Main cytogenicity test:

Metabolic activation	Duration of exposure (Fixation time)	Concentrations[§]	Replicates
- S9 mix	4 h (20 h)	191.25, 382.5[*], 765[*] and 1530[*#] µg/mL	Duplicate
- S9 mix	20 h (20 h)	191.25, 382.5[*], 765[*] and 1530[*#] µg/mL	Duplicate
+ S9 mix	4 h (20 h)	191.25, 382.5[*], 765[*] and 1530[*#] µg/mL	Duplicate

* Samples analysed for chromosomal aberrations

[#]: highest concentration, corresponding to 10 mM

[§]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.45.

B. STUDY DESIGN AND METHODS

Dates of experimental work: 08 Mar – 17 Apr 2007

Finalisation date: 18 Jun 2007

2. Preliminary cytotoxicity test:

In a preliminary cytotoxicity test, human peripheral lymphocytes were treated with the test item at concentrations in the range of 0.153 – 1530 µg/mL in the presence and absence of S9 mix. The test was conducted under the same conditions as in the main cytogenicity experiment. Before treatment, cells were grown for 44-48 hours in a medium supplemented with 1% phytohemagglutinin (PHA) in order to induce cell division prior to exposure. A single cell culture per condition was exposed for 4 hours in the presence and absence of S9 mix and for 20 hours in the absence of S9 mix. All cells were harvested 20 hours after start of exposure. Two hours prior to harvest, 0.1 µg/mL colcemid was added to the cultures to arrest cells in metaphase. At harvest, the cells were collected by centrifugation, treated with hypotonic potassium chloride (75 mM), fixed and stained. The number of cells in mitosis was determined per 500 cells scored in order to evaluate the test item effect on mitotic index.

3. Main cytogenicity test:

Treatment:	Following cell culture establishment (including induction of cell division), duplicate cultures per condition were exposed to the test item, solvent (water) or positive control (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) in the presence and absence of metabolic activation. The cells were exposed for 4 hours in the presence and absence of S9 mix and for 20 hours in the absence of S9 mix. Cells of the 4-hour exposure period were washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS) following treatment, re-fed with culture medium and returned to the incubator for 16 further hours. For all conditions, chromosome preparations were made 20 hours after start of exposure.
Spindle inhibition:	During the last 2 hours of the culture period, cell division was arrested by addition of 0.1 µg/mL colchicine each culture.
Cell harvest:	The cell cultures were harvested by centrifugation, the pellets were re-suspended in 75 mM potassium chloride and swollen for 20 minutes at 37 ± 1 °C. Afterwards, the cells were gently mixed and approx. 0.5 mL of fixative (methanol : glacial acetic acid, 3:1, v/v) was added to each tube. Cells were collected by centrifugation, washed twice in fixative and stored in fixative until preparation.
Slide preparation:	Fixed cells were centrifuged and re-suspended in 0.1 – 0.3 mL of the supernatant fixative above the cell pellet. One or two drops of cell suspension were placed on glass slides and allowed to air-dry. Dried slides were coded, stained with 5 % Giemsa, air-dried and permanently mounted.
Metaphase analysis:	A total number of 200 metaphase cells per condition (100 per duplicate treatment condition) were examined by microscopy. Only metaphase cells with 46 centromers were examined. In case the percentage of aberrant cells reached a significant level (≥ 10 %) before 100 cells were scored, less metaphases were investigated. Chromatide-type and chromosome-type aberrations were recorded, including breaks, exchange figures and complex re-arragements. Chromatid and isochromatid gaps are recorded but not included in the analysis. In addition, pulverised chromosomes and cells and severely damaged cells were recorded. The percentage of polyploidy and endoreduplicated cells was evaluated per 100 cells.
Cytotoxicity:	The mitotic index of each culture was calculated based on the number of cells in metaphase observed per 500 cells scored.

4. Statistics

Statistical analysis of the percent aberrant cells was performed using the Fisher's Exact test. Fisher's Exact test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's Exact test at any test substance dose level, the Cochran-Armitage test was used to measure dose-responsiveness.

5. Acceptance criteria

The chromosome aberration test was considered acceptable if the following criteria were met:

- The frequency of cells with structural chromosome aberrations in the vehicle control was within the laboratories historical range.
- The percentage of cells with chromosome aberrations in the positive control was statistically increased ($p \leq 0.05$) relative to the solvent control.

6. Evaluation criteria

A test substance was considered positive (clastogenic) in the chromosome aberration test if the following criteria were met:

- The percentage of cells with aberrations was increased in a dose-related manner with one or more concentrations being statistically elevated relative to the solvent control group ($p \leq 0.5$).
- Values that were statistically significant but did not exceed the range of historical solvent control data

were judged as not biologically significant.
A test substance not demonstrating a statistically significant increase in aberrations was concluded to be negative.

II. RESULTS AND DISCUSSION

1. ANALYTCAL DETERMINATIONS

Analytical determinations of the test substance in the solvent were performed for the formulations 1.9125, 3.825 and 15.30 mg/mL. The concentrations determined were 111.9 %, 110.8 % and 107.8 % of the nominal concentration, respectively, which were within the accepted range of 85 – 115 % of the target concentration. According to the analytical analysis conducted by the sponsor (GLP compliant), the formulations proved to be stable for 5 hours at room temperature.

2. CYTOTOXICITY

Preliminary cytotoxicity test

In the preliminary cytotoxicity test, substantial cytotoxicity (at least 50 % reduction in mitotic index when compared to the solvent control) was not observed for any treatment schedule, up to the highest tested concentration of 1530 µg/mL, neither in the presence, nor in the absence of S9 mix. The osmolality of the highest test substance concentration in treatment medium did not exceed the osmolality of the solvent by more than 20 % and was therefore considered acceptable. The pH of the highest test substance concentration was 7.0. Based on these results, 1530 µg/mL (=10 mM) was chosen as the highest test item concentration for the main cytogenicity study.

Main mutagenicity test

In the main mutagenicity test, cytotoxicity evident as a reduced mitotic index of ≥ 50 % was not observed for any treatment schedule or any concentration, neither in the presence, nor in the absence of S9 mix.

3. SOLUBILITY

Precipitation of the test item in solvent or culture medium was not observed for any treatment schedule, neither in the presence, nor in the absence of metabolic activation.

4. CYTOGENICITY (MAIN MUTAGENICITY TEST)

There was no statistically significant increase in the number of cells with structural or numerical chromosomal aberrations observed for any treatment schedule, neither in the presence nor in the absence of metabolic activation. The percentage of aberrant cells in the test substance-treated groups was comparable to those of solvent controls at any dose level. The number of average aberrations per cell observed for the highest test item concentration after 4 hours of exposure in the presence of S9 mix (0.005 ± 0.071) was increased when compared to those of the solvent controls (0.0 ± 0.0). The value observed for the test item is explained by a single chromosome aberration of gap-type, which is not included in the assessment of total aberration frequency. As the percentage of structural aberrant cells was within the range of historical control data, the observation was considered not relevant. The frequency of chromosomal aberrations observed for the solvent and positive controls was within the range of the laboratory's historical control data. The positive controls (mitomycin C without S9 mix and cyclophosphamide with S9 mix) induced a statistically significant increase in the number of structural chromosome aberrations, demonstrating the functionality of the metabolic activation system and proving the validity of the test.

Table B.6.8.1.2.3.2-1: Results of the cytogenicity test with [(Acetylamino)methyl]phosphonic acid (IN-EY252) ([redacted], 2007)

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity					Mitotic index [%]
			% structural aberrant cells	% numerical aberrant cells	Total number of structural aberrations		Average aberrations / cell	Judge	
					incl. gaps	excl. gaps			
Without metabolic activation; 4-hour treatment and 20-hours sampling									
Solvent									

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Mitotic index [%]
			% structural aberrant cells	% numerical aberrant cells	Total number of structural aberrations		Average aberrations / cell	Judge	
					incl. gaps	excl. gaps			
Water	0	200	0.0	0.0	0.0	0.0	0.000	negative	12.1
HCD[#] mean ± SD			0.0 ± 0.1	0.0 ± 0.2					
range			0.0 - 1.0	0.0 - 1.5					
Test item [µg/mL]									
	382.5	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	11.5
	765	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	11.1
	1530	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	10.6
Positive control									
MMC	0.6	100 (structural aberrations); 200 (numerical aberrations)	**18****	0	**21****	**21****	**0.210 ± 0.478****	positive	9.0
HCD[#] mean ± SD			17.8 ± 5.8	0.1 ± 0.3					
range			6.0 - 40.0	0.0 - 2.0					
Without metabolic activation; 20-hour treatment and sampling									
Solvent									
Water	0	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	11.9
HCD[#] mean ± SD			0.0 ± 0.1	0.0 ± 0.2					
range			0.0 - 1.0	0.0 - 1.5					
Test item [µg/mL]									
	382.5	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	11.3
	765	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	10.9
	1530	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	9.7
Positive control									
MMC	0.3	100 (structural aberrations); 200 (numerical aberrations)	**16****	0	**18****	**18****	**0.180 ± 0.435****	positive	8.2
HCD[#] mean ± SD			17.8 ± 5.8	0.1 ± 0.3					
range			6.0 - 40.0	0.0 - 2.0					
With metabolic activation; 4-hour treatment, 20-hours sampling time									

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Mitotic index [%]
			% structural aberrant cells	% numerical aberrant cells	Total number of structural aberrations		Average aberrations / cell	Judge	
					incl. gaps	excl. gaps			
Solvent control									
Water	0	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	8.2
HCD# mean ± SD			0.0 ± 0.1	0.0 ± 0.2					
range			0.0 - 0.5	0.0 - 1.5					
Test item [µg/mL]									
	382.5	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	7.2
	765	200	0.0	0.0	0.0	0.0	0.000 ± 0.00	negative	6.9
	1530	200	0.5	0.0	1.0	1.0	0.005 ± 0.071	negative	7.9
Positive control									
CP	20	100 (structural aberrations); 200 (numerical aberrations)	**19****	0	**23****	**23****	**0.230 ± 0.510****	positive	7.0
HCD# mean ± SD			17.3 ± 5.5	0.1 ± 0.4					
range			9.0 - 40.0	0.0 - 2.5					

HCD#: Historical control data from the laboratory obtained in 2003 – 2005.
MMC: Mytomicin C, positive control without S9 mix; CP: cyclophosphamide, positive control with S9 mix
Mitotic index: Number of mitotic figures x 100 / 500 cells counted
% of aberrant cells: numerical includes polyploidy and endoreduplicated cells; structural excludes cells with only gaps
** $p \leq 0.01$ using Fisher's Exact Test

III. CONCLUSIONS

Based on the experimental findings there is no evidence for an induction of structural or numerical chromosome aberrations by IN-EY252, metabolite of glyphosate, in peripheral human lymphocytes, neither in the presence nor in the absence of metabolic activation.

Assessment and conclusion by applicant:

In the present study, [(Acetylamino)methyl]phosphonic acid was negative for cytogenicity (chromosome aberrations *in vitro*) in peripheral human lymphocytes with and without metabolic activation.
The test was performed under GLP conditions and in accordance with OECD guideline 473 (1997).
There were only minor deviations when compared to OECD 473 (2016). The number of metaphases investigated was only 200, which was the number to be investigated recommended by the previous OCED 473 (1997). The study is therefore considered valid.

Assessment and conclusion by RMS:

It is agreed with the applicant that, in this test, N-acetyl AMPA was negative for cytogenicity (chromosome aberrations *in vitro*) in peripheral human lymphocytes with and without metabolic activation. The study is considered acceptable but with restrictions (reliable with restrictions) due to the deviation in number of scored metaphases. It is noted, however, that the study was conducted according to the applicable guideline (OECD 473, 1997) when conducting the study. The study was not available for the previous assessment (RAR, 2015).

Study 3

Data point	CA 5.8.1/036
Report author	[redacted]
Report year	2007
Report title	IN-EY252: In Vitro Mammalian Cell Gene Mutation Test (CHO/HGPRT)
Report No	DuPont-22224
Document No	-
Guidelines followed in study	OECD 476 (1997), US EPA OPPTS 870.5300, EC Commission Directive 2000/32/EC Annex 4E-B17 (2000), JMAFF (1985)
Deviations from current test guideline	The newly introduced cytotoxicity parameter RS (relative survival) in OECD 476 (2016) and the adjusted cloning efficiency were not re-calculated, since no data on the number of cells after treatment were provided. In the current study, cytotoxicity was evaluated based on cloning efficiency after treatment (CE_1, survival) and after selection (CE_2, viability), in accordance with the previous guideline version. Historical control data for the negative control were reported without 95 % control limits. In addition, acceptability and evaluation criteria specified in the study report were inconsistent with those specified in the current guideline. In particular, the RMS notes that the number of treated cells (assumed to be approximately 1 x 10^6 cells) and cells cultivated during expression and mutant selection phases ($\leq 10^6$ cells) are neither in agreement with the OECD guideline applicable when the study was conducted (at least 10^6 cells at each stage in the test [OECD, 1997]) nor with the current OECD guideline (20 x 10^6 cells at treatment and at least 2 x 10^6 cells during expression and mutant selection [OECD, 2016]).
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 1) **Conclusion AGG:** The study is considered to be acceptable but with restrictions (reliable with restrictions) due to the deviations noted above.

Executive summary

[(Acetylamino)methyl]phosphonic acid was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese hamster ovary (CHO K_1) cells. Duplicate cultures were exposed to the test item, solvent (water) and positive controls (ethylmethane sulfonate for cultures without S9 mix and benzo(a)pyrene for cultures with S9 mix) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Based on the results of a preliminary toxicity test, in which no substantial cytotoxicity was observed up to 1531 µg/mL (corresponding to 10 mM), the concentrations for the main mutagenicity study were selected. [(Acetylamino)methyl]phosphonic acid concentrations in the range of 100 – 1531 µg/mL were applied. After 5 hours of exposure with and without S9 mix, the cells were incubated for 7 days to allow expression of the mutant phenotype. The expression period was followed by a selection period, in which the cells were cultivated in 6-thioguanidine-enriched medium for 10 days. Cytotoxicity was assessed as relative survival and relative viability after the expression and selection period, respectively.

There was no precipitation of the test item in solvent and in culture medium, neither in the presence nor absence of S9 mix. Substantial cytotoxicity was not observed for any condition at any concentration. The relative cloning efficiency at the highest concentration of 1531 µg/mL was 67 % without S9 mix and 83 % with S9 mix.

There was no increase in the number of mutant colonies upon treatment with the test substance, neither in the presence nor in the absence of metabolic activation. For all conditions and at all dose levels, mutant frequencies were below the minimum value for a positive response.

Mutant frequencies of the control cultures remained within the range of the laboratories historical control data. The positive control mutagens ethylmethane sulfonate and benzo(a)pyrene induced mutant frequencies that were at least 3-fold above those of vehicle controls, demonstrating the functionality of the S9 mix and the sensitivity of the test.

Under the conditions of the test, there was no evidence for gene mutation in mammalian cells *in vitro*, neither in the presence nor in the absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: [(Acetylamino)methyl]phosphonic acid

Identification:	IN-EY252
Description:	White solid
Lot/Batch number:	IN-EY252-002
Purity:	72%
Stability of test compound:	The stability of the test item at storage conditions (with a desiccator) was guaranteed until the expiry date 13 Apr 2010. The stability of the test substance in vehicle was confirmed by analytical methods.

2. Control material:

Negative control:	The negative control was actually the solvent control.
Solvent (vehicle) control:	Water
Positive control:	- S9 mix: Ethylmethane sulfonate (EMS), 0.2 µL/mL in DMSO + S9 mix Benzo(a)pyrene (BaP), 4 µg/mL in DMSO

3. Metabolic activation:

S9 mix was purchased from [redacted]. The liver homogenate was produced from the livers of male Sprague-Dawley rats that were induced with Aroclor 1254. Immediately prior to use the S9 liver homogenate was thawed and mixed with co-factors as follows:

S9 mix component	**Concentration**	**Unit**
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organism:

Chinese hamster ovary ($CHO\text{-}K_1$) cells were obtained from American Type Culture Collection (ATCC) in Manassas, Virginia, USA. The cell stocks were stored in liquid nitrogen and routinely tested for mycoplasma contamination. Cells used in the mutation assay were within three to four subpassages from cleansing in pre-treatment medium supplemented with hypoxanthine, aminopterin and thymidine (HAT) in order to assure karyotypic stability.

5. Cell culture media:

Pre-treatment medium (HAT medium):	Ham's F12 medium supplemented with hypoxanthine, aminopterin and thymidine
Cultivation medium (F12FBS5-Hx):	Ham's F12 medium, supplemented with 5 % dialysed fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin
Treatment medium (± S9):	Cultivation medium (F12FBS5-Hx)
Selection medium:	Cultivation medium (F12FBS5-Hx) supplemented with 10 µM 6-thioguanidine (6TG)
Incubation:	At 37.5 ± 2 °C in a humidified atmosphere of 5 ± 2 % CO_2
Locus examined:	Hypoxanthin guanine phosphoribosyltransferase (HGPRT)

Test concentrations and number of replicates:

Preliminary cytotoxicity and mutagenicity test:

Metabolic activation	**Duration of exposure**	**Concentrations[§]**	**Replicates**
± S9 mix	5 h	5, 10, 25, 50, 100, 250, 500, 1000 and 1531 µg/mL	Triplicate plates from a single culture

[§]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.389.

Main gene mutation test:

Metabolic activation	Duration of exposure	Concentrations[§]	Replicates
± S9 mix	5 h	100, 250, 500, 1000 and 1531 µg/mL	Triplicate plates from duplicate cultures

[§]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.389.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 30 May – 12 Sep 2007
Finalisation date: 25 Sep 2007

2. Preliminary cytotoxicity and mutagenicity test:
A preliminary test was performed to identify suitable dose levels for the main mutagenicity study. Cell cultures were established identically to the performance in the main mutagenicity test described below. The cells were exposed to nine test substance concentrations in the range of 5 – 1531 µg/mL for 5 hours in the presence and absence of S9 mix at 37 ± 2 °C. The highest concentration was equivalent to a 10 mM concentration. Following exposure, the cells were washed with Hank's Balanced Salt Solution (HBSS), re-incubated in fresh culture medium for approx. 20 hours, harvested and plated for determination of cell survival (cloning efficiency 1). After incubation for 8 days, the formed colonies were fixed, stained and counted.
Based on the results of the preliminary test, the test item concentrations for the main mutagenicity test were selected.

3. Main mutation assay:
Pre-treatment of cells:
For each test group, 5 x 10^5 cells were seeded into 25 cm^2 flasks and incubated for 16 - 24 hours prior to treatment. The RMS notes that the number of cells used at treatment was not determined. Assuming a doubling time of around 20 hours, approximately 1 x 10^6 cells would have been treated which is not in line with the OECD guideline ('see deviations').

Treatment:
On the day of treatment, the medium was exchanged. For treatment in the presence of S9 mix, 20 % of the medium were replaced by S9 mix. Duplicate cultures were exposed to the test item, vehicle (water) and positive controls (0.2 µL/mL ethylmethane sulfonate without S9 mix and 4 µg/mL benzo(a)pyrene with S9 mix) for 5 hours in the presence and absence of metabolic activation at 37 ± 2 °C. Test item concentrations ranged from 100 – 1531 µg/mL. Following exposure, the cells were washed with Hank's Buffered Salt Solution (HBSS) and the culturing was continued for another 18 – 24 hours. After the incubation period, the cells were trypsinised and counted. Triplicates of 100 cells/ 60 mm dish were seeded for the determination of survival (cloning efficiency 1) and duplicate cultures with ≤ 10^6 cells/ 100 mm dish were seeded for the expression of the mutant phenotype.

Expression period:
After treatment, duplicate cultures with ≤ 10^6 cells/ 100 mm dish were seeded and sub-cultured at 2 - 3 day intervals for a 7-day expression period. The RMS notes that the number of cells cultivated during the expression phase is not in agreement with the OECD guideline (see 'deviations'). After the expression period, each culture was divided. Triplicates of 100 cells/ 60 mm dish were seeded for the determination of viability (cloning efficiency 2) and five culture dishes with 2 x 10^5 cells/ 100 mm dish were seeded for the selection of mutants.

Selection period:
For the selection of mutants, five culture dishes with 2 x 10^5 cells/ 100 mm dish were seeded in medium enriched with 10 µM 6-thioguanidine (selection medium). The RMS notes that the number of cells cultivated during the mutant selection phase is not in agreement with the OECD guideline (see 'deviations'). After an incubation period of 10 days, the colonies were fixed, stained and counted.

4. Cytotoxicity:
Cloning efficiency CE_1 (survival)
The survival (cloning efficiency 1) of [(Acetylamino)methyl]phosphonic acid-treated cells relative to solvent controls was determined in parallel to the mutagenicity test. At the end of the exposure period, a sample of each cell culture was collected to assess survival of the cells. Triplicates of 100 cells/60 mm dish were seeded and

incubated for 7 days. Afterwards, the colonies were fixed, stained and counted.

Cloning efficiency CE_2 (viability)
The viability (cloning efficiency 2) was determined in parallel to the selection of mutants. After the expression period, triplicates of 100 cells/60 mm dish were seeded in medium without 6-thioguanine to assess cell viability. After an incubation period of 10 days, the developed colonies were fixed, stained and counted.

5. Evaluation:
Cytotoxicity (cloning efficiency CE)
The number of colonies divided by the number of cells plated was calculated for each sample. The absolute cloning efficiency was determined for each test group, as well as the relative cloning efficiency in comparison to the solvent control group.

CE_1 (survival)
The cytotoxicity of the test substance after the exposure period was determined for each test group and is indicated as absolute and relative cloning efficiency (CE_1 and RCE_1, respectively).

CE_2 (viability)
The cytotoxicity of the test substance at the end of the expression period was determined for each test group and is given as absolute and relative cloning efficiency (CE_2 and RCE_2, respectively).

The cloning efficiency (CE, %) was calculated for each test group as follows:

$$CE_{absolute} = \frac{\text{Total number of colonies}}{\text{Total number of cells plated}} \times 100$$

$$RCE_x = \frac{CE_{absolute} \text{ of the test group}}{CE_{absolute} \text{ of the vehicle control group}} \times 100$$

Mutant frequency (MF)
The cloning efficiency of mutant colonies in selective medium divided by the cloning efficiency in non-selective medium measured for the same culture at the time of selection was calculated for each sample.

Uncorrected mutant frequency:
The uncorrected mutant frequency (MF_{uncorr}) was calculated for each test group as follows:

$$\text{MF uncorrected} = \frac{\text{Total number of mutant colonies}}{\text{Number of seeded cells}} \times 10^6$$

Corrected mutant frequency
The corrected mutation frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$\text{MF corrected} = \frac{\text{MF uncorrected}}{CE_2} \times 100$$

6. Statistics:
Statistical analysis was not performed.

7. Acceptance criteria:
The test was considered valid if the following criteria were met:

- The cloning efficiency of the vehicle control was > 50 % with a spontaneous mutant frequency within the range of the laboratories historical control data (0-25 mutants per 10^6 clonable cells).
- The mutant frequency of the positive control was at least 3-fold as compared to the concurrent vehicle control and exceeded 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data).
- At least 4 analysable concentrations showing mutants were required for evaluation.

1. 8. Evaluation criteria:
A test item was judged positive for gene mutation in mammalian cells if the following criteria were met:

- A mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the

laboratories historical control data) was observed at 2 or more consecutive concentrations of the test substance when compared to the solvent control.
- The observed increase in mutant frequency was accompanied by a concentration-related increase.
- A mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at the highest concentration only.

The test item was judged equivocal if a mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at any dose level other than the highest concentration.
The test item was judged negative for gene mutation in mammalian cells if a mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was not observed at any dose level.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS
Analytical determinations were performed using a high performance liquid chromatography (HPLC) with UV/Vis detection. The test item formulations were ± 7 % (preliminary toxicity test) and ± 5 % (main mutation assay) of the nominal concentrations and therefore within the acceptable range (± 10 % of the nominal concentration). There was no test substance in the 0 mg/mL sample. Stability of the test item in vehicle was confirmed for 23 hours at room temperature.

2. CYTOTOXICITY
In the preliminary cytotoxicity test, no cytotoxicity, evident as a cloning efficiency of ≤ 50 %, was observed. The relative cloning efficiency at the highest test item concentration (1531 µg/mL) was 59 % in the absence of S9 mix and 308 % in the presence of S9 mix. Osmolality and pH measurements of test substance in medium at 1531 µg/mL were found to be within an acceptable range (≤ 20 %). Based on these findings, the concentrations for the main mutagenicity study were selected to be in the range of 100 – 1531 µg/mL, corresponding to 0.65 – 10 mM.
In the main mutagenicity experiment, no substantial cytotoxicity was observed at any dose level, neither in the presence nor absence of S9 mix.

SOLUBILITY
There was no precipitation observed up to the highest concentration tested, neither in the presence, nor absence of metabolic activation.

3. MUTANT FREQUENCY
In a first trial, a high spontaneous mutant frequency was observed for the solvent controls in both, cultures with and without S9 mix (data not shown). The test was therefore considered invalid and repeated.
In the second trial, there was no increase in the number of mutant colonies upon treatment with [(Acetylamino)methyl]phosphonic acid, neither in the presence nor in the absence of metabolic activation. For all conditions and at all dose levels, mutant frequencies were below 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) and no dose-response relationship was evident.
Mutant frequencies of the solvent control cultures remained within the range of the laboratory's historical control data. The positive control mutagens ethylmethane sulfonate and benzo(a)pyrene induced mutant frequencies that were at least 3-fold above those of vehicle controls, demonstrating the functionality of the S9 mix and the sensitivity of the test.

Table B.6.8.1.2.3.3-1: Results of the HGPRT gene mutation assay in mammalian cells with [(Acetylamino)methyl]phosphonic acid (IN-EY252) ([redacted], 2007)

Test group	Total number of mutant colonies[$]	Mutant frequency (per 10^6 cells)	Cloning efficiency			
			CE_1 (survival)		CE_2 (viability)	
		corr.[#]	abs.	rel. [%]	abs.	rel.[§] [%]
Without metabolic activation; 5-hour exposure period						
Solvent						
Water	28	24.7	0.97	100	0.57	100
HCD mean ± SD		6.1 ± 6.6				

Test group	Total number of mutant colonies[$]	Mutant frequency (per 10^6 cells)	Cloning efficiency			
			CE_1 (survival)		CE_2 (viability)	
		corr.[#]	abs.	rel. [%]	abs.	rel.[§] [%]
range		*0.0 - 24.0*				
Test item [µg/mL]						
100	26	19.3	1.12	116	0.67	118
250	34	22.4	0.89	92	0.76	133
500	25	18.5	0.98	101	0.68	119
1000	13	8.7	0.85	87	0.75	132
1531	14	10.5	0.80	82	0.67	118
Positive control [µL/mL]						
EMS 0.2	267	188.0	0.66	68	0.71	125
HCD mean ± SD		216.0 ± 148.5				
range		*75.9 - 880.1*				
With metabolic activation; 5-hour exposure period						
Solvent						
Water	34	26.2	0.40	100	0.65	100
HCD mean ± SD		5.5 ± 5.4				
range		*0.0 - 19.0*				
Test item [µg/mL]						
100	24	20.9	0.59	146	0.57	88
250	22	16.1	0.61	152	0.68	105
500	21	16.6	0.83	206	0.63	97
1000	27	27.2	0.86	212	0.50	77
1531	29	17.5	0.77	192	0.83	128
Positive control [µg/mL]						
(B(a)P) 4.0	102	106.3	0.32	79	0.48	74
HCD mean ± SD		206.3 ± 118.5				
range		*89.7 - 379.8*				

= correction on the basis of the absolute cloning efficiency 2 at the end of the expression period
$: number of mutant colonies 10 days after seeding 2 x 105 cells/100 mm dish, total of 5 cultures
§: calculated with total cloning efficiency data (solvent control = 100 %)
HCD: Historical control data, generated in the laboratory from 1989 – 2005. Data include all control solvents or diluents. The RMS notes that the historical control data covers quite a long period (up to 18 years prior to conduct of the study). However, considering the clear negative response in the test item groups this is not considered to be critical.

III. CONCLUSIONS

Based on the experimental findings and under the conditions of the test, [(Acetylamino)methyl]phosphonic acid did not induce gene mutations in the HGPRT locus of CHO-K_1 cells, neither in the presence nor in the absence of metabolic activation and is therefore considered negative for mutagenicity in mammalian cells *in vitro*.

Assessment and conclusion by applicant:

In the present study, [(Acetylamino)methyl]phosphonic acid was negative for mutagenicity at the HGPRT locus in CHO-K_1 cells with and without metabolic activation.

The study was conducted in compliance with GLP and according to OECD guideline 476 (1997). There were only minor deviations when compared to OECD 476 (2016), which were considered to not compromise the validity of the study. The study is therefore considered valid.

Assessment and conclusion by RMS:

It is agreed with the applicant that, in this study, [(acetylamino)methyl]phosphonic acid was negative for mutagenicity at the HGPRT locus in CHO-K_1 cells with and without metabolic activation. However, due to the deviations noted at the beginning of the study summary, the study is considered to be acceptable but with restrictions (reliable with restrictions) only.
The study was not available for the previous evaluation (RAR, 2015).

Study 4

Data point	CA 5.8.1/037
Report author	[redacted]
Report year	2007
Report title	IN-EY252: Mouse Bone Marrow Micronucleus Test
Report No	[redacted]-22226
Document No	NA
Guidelines followed in study	OECD 474 (1997), US EPA OPPTS 870.5395 (1998), EEC Directive 2000/32/EC B12 (2000), JMAFF 12 Nousan (2000)
Deviations from current test guideline	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. However, in the present study only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity observed Dose levels included limit concentrations specified in the current guideline. However, this is not sufficient to conclude that bone marrow exposure occurred and that the lack of an increase in mPCE represents a true negative result. In addition, no 95 % control limits were reported for the historical control data.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 1) **Conclusion AGG:** The study is considered to be acceptable but with restrictions (reliable with restrictions).

Executive summary
[(Acetylamino)methyl]phosphonic acid was tested for its potential to induce micronuclei in male and female Crl:CD1 (ICR) mice. Based on the results of a preliminary toxicity study, dose levels for micronucleus tests were selected. Groups of 5 mice per sex per dose level were administered a single dose of 500, 1000 or 2000 mg/kg bw by oral gavage. For the high dose group, 2 additional mice/sex/sampling time point were treated and served as backup (scoring of micronuclei was not performed). Similar constituted groups received the vehicle (deionised water) or the positive control (30 mg/kg bw cyclophosphamide).
Body weights of the animals were collected prior to treatment and 24 and 48 hours post dosing. The animals were observed for clinical signs of toxicity and mortality 1, 3.5, 24 and 48 hours after treatment. About 24 and 48 hours after dosing, the animals of the test item-treated and vehicle control groups were sacrificed and bone marrow smears were prepared. For animals of the positive control group, bone marrow was sampled 24 hours after dosing. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. In addition, the proportion of PCE among 1000 erythrocytes, expressed as ratio PCE / normochromatic erythrocytes (NCE) was determined.
Treatment with [(Acetylamino)methyl]phosphonic acid induced no mortality in the animals and no clinical signs of toxicity were noted at any dose level. In addition, body weight and body weight gain were not affected in any dose group at any sampling time point. Based on the ratio of PCE / NCE, there was no evidence for bone marrow toxicity.
There was no statistically significant and no biologically relevant increase in the frequency of micronucleated PCE (mPCE) when compared to vehicle control animals at any dose level and for any sampling time.
Incidences of mPCE in solvent and positive control animals were within the range of the laboratory's historical control data and demonstrated the validity and sensitivity of the test system.
Based on the experimental findings of the present study and under the conditions of the test, the test item did not induce micronuclei in the bone marrow of male and female mice *in vivo*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: [(Acetylamino)methyl]phosphonic acid

Identification:	IN-EY252
Description:	White solid
Lot/Batch #:	IN-EY252-002
Purity:	72 %
Stability of test compound:	The stability of the test item at storage conditions (with a desiccator) was guaranteed until the expiry date 13 Apr 2010. The stability of the test substance in vehicle was confirmed by analytical methods.
Solvent (vehicle) used:	Deionised water

2. Control materials

Solvent (vehicle) control:	Deionised water
Positive control:	Cyclophosphamide, 30 mg/kg bw in deionised water

3. Test animals:

Species:	Mouse
Strain:	Crl:CD1 (ICR)
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	Approx. 7 weeks
Mean weight at dosing:	28.2 – 37.5 g (males) and 21.4 – 27.6 g (females)
Acclimation period:	At least 5 days
Diet/Food:	LLC Certified Rodent LabDiet® 5002 (PMI® Nutrition International), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually

1. **Environmental conditions:**

Temperature:	18 - 26 °C
Humidity:	30 - 70 %
Air changes:	Not specified
Photoperiod:	12-hour light and dark cycle

Test concentrations and treatment groups:

Preliminary toxicity study

Dose levels:	2000 mg/kg bw
Concentrations:	Not specified
Dose volume:	Not specified
Number of animals:	3 males
Route of administration:	Oral gavage

Main micronucleus test

Dose levels:	500, 1000 and 2000 mg/kg bw (Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor)
Concentrations:	50, 100 and 200 mg/mL
Dose volume:	10 mL/kg bw
Number of animals:	5/sex/group/sampling time point and additional 2/sex/sampling time point as backup for the high dose group (additional animals were not used for scoring of micronuclei)
Route of administration:	Oral gavage

B. STUDY DESIGN AND METHODS

Dates of experimental work: 22 May – 23 Jul 2007
Finalisation date: 07 Sep 2007

1. Animal assignment and treatment:

Preliminary toxicity study:
In a preliminary range-finding test 3 male animals were administered a single dose of 2000 mg/kg bw by oral gavage. The animals were observed for clinical signs of toxicity and mortality immediately after dosing and daily for two days. Based on the results of the preliminary toxicity study, the dose levels for the micronucleus test were selected.

Main micronucleus test:
Groups of 5 mice per sex and dose level and sampling time point were administered a single dose of 500, 1000 or 2000 mg/kg bw. For the high dose group, 2 additional mice/sex/sampling time point were treated and served as backup (scoring of micronuclei was not performed). The test substance was dissolved in deionised water and administered by oral gavage at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of 5 mice/sex received the vehicle (deionised water) or the positive control (30 mg/kg bw cyclophosphamide).
Individual body weights were collected prior to treatment and 24 and 48 hours post dosing. The animals were observed for clinical signs of toxicity and mortality 1, 3 - 5, 24 and 48 hours after treatment. About 24 and 48 hours after dosing, the animals of the test item-treated and control groups were sacrificed by CO_2 asphyxiation and bone marrow samples were generated. Animals of the positive control group were sacrificed 24 hours after administration only.

2. Slide preparation:

After sacrifice, both femurs of the animals were removed, and marrow was aspirated with the aid of a syringe containing fetal bovine serum (FBS). After centrifugation, the pellet was re-suspended in FBS and a small drop was placed on pre-cleaned microscope slides. Smears were made using a Mini Prep® blood smearing instrument. At least 3 slides were prepared for each animal. The slides were air-dried, fixed in methanol and stained in acridine orange.

3. Slide evaluation:

Slides were coded and evaluated by fluorescent microscopy. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. Cells containing more than one micronucleus were scored as single micronucleated PCE (mPCE). In addition, the proportion of PCE among 1000 erythrocytes, expressed as ratio PCE / normochromatic erythrocytes (NCE) was determined.
If no increase in the incidence of mPCE was observed at the 24-hour sampling time point at any dose level, slide evaluation for the 48-hour sampling was performed for animals of the high dose and control group only.

4. Statistics:

Data for the amount of micronucleated polychromatic erythrocytes (mPCE) among 2000 erythrocytes and the ratio of PCE among normochromatic erythrocytes (NCE) among 1000 total erythrocytes were transformed prior to analysis using an arcsine square root or Freeman-Tukey function. Transformed data for PCE and MNPCE frequencies were analyzed separately for normality of distribution and equal variance using the Shapiro-Wilk and Levene's tests, respectively.

For those data that were normally distributed and had equal variance, parametric statistics (e.g. analysis of variace (ANOVA) and Dunnett's test) were performed using the transformed data. For those data that were normally distributed but had unequal variance, a robust ANOVA and unequal-variance Dunnett test was done. For those data that were not normally distributed, nonparametric statistics (e.g., Kruskal-Wallis and Dunn's tests) utilizing non-transformed data was performed. The individual animal was considered the experimental unit. All data analyses was one-tailed and conducted at a significance level of 5 %.

5. Acceptance criteria:

The study was considered valid if the following criteria were met:

- In the vehicle control group, the mean frequency of mPCEs did not exceed 10 per 2000 PCEs.
- In the vehicle control group, the mean frequency of mPCEs was within the range of the laboratories historical control range.
- The positive control induced a statistically significant increase in the frequency of mPCE as compared to the vehicle control group ($p \leq 0.05$).

6.

7. Evaluation criteria:

The test item was considered positive if the following criteria were met:

- The group mean number of micronucleated polychromatic erythrocytes (mPCE) was statistically significantly increased at one or more concentrations when compared to the concurrent vehicle control value.
- An accompanying statistically significant dose-response increase in mPCE was observed.

The test item was considered negative if the following criteria were met:

- There was no statistically significant, dose-related increase in the group mean mPCE above the concurrent vehicle control at any concentration.
- The mPCE values were within reasonable limits of the recent (past 3 years) laboratory historical control range.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS

The dosing formulations were analysed by high performance liquid chromatography (HPLC) and UV/Vis detection. The data indicated that the achieved concentration of test substance in vehicle was ± 5 % of the nominal concentration, which was considered acceptable. Stability of the test substance in vehicle was confirmed for 5 hours at room temperature.

2. PRELIMINARY TOXICITY STUDY

Data on clinical signs of toxicity in the preliminary toxicity study were not included.

3. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

Clinical signs of toxicity were not observed at any time point or at any dose level.

Body weight and body weight gain:

There were no significant changes in body weight or body weight gain in either male or female animals of any test group.

Evaluation of bone marrow slides:

Upon treatment with the test substance at any dose level and sampling time point, the ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) was not affected, indicating that no bone marrow toxicity was

evident. A depression in PCE frequency was neither observed in male or female animals from the positive control group. Therefore, there is no direct proof of bone marrow exposure.
In addition, there was no statistically significant and no biologically relevant increase in the frequency of mPCE when compared to vehicle control animals at any dose level and for any sampling time.
Incidences of mPCE in solvent and positive control animals stayed within the range of the laboratory's historical control data and proved the validity and sensitivity of the test system.

Table B.6.8.1.2.3.4-1: Summary of genotoxicity data obtained with [(Acetylamino)methyl]phosphonic acid (IN-EY252) in the micronucleus test in mice ([redacted], 2007)

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD / 2000 PCE	PCE / NCE	mPCE ± SD / 2000 PCE	PCE / NCE
Solvent control						
Deionised water	/	24 h	1.2 ± 1.3	1.149 ± 0.187	3.0 ± 1.6	1.362 ± 0.400
	/	48 h	1.2 ± 0.8	1.146 ± 0.298	4.2 ± 1.6	1.306 ± 0.330
HCD mean ± SD	/	not specified	2 ± 2	1.27 ± 0.48	3 ± 2	1.35 ± 0.47
range	/		*0 - 8*	*0.198 - 3.184*	*0 - 10*	*0.144 - 2.731*
Test item						
Test item	500	24 h	2.8 ± 2.5	1.367 ± 0.201	1.6 ± 1.5	1.533 ± 0.350
	1000	24 h	1.6 ± 1.8	1.376 ± 0.322	1.6 ± 0.9	1.406 ± 0.228
	2000	24 h	1.8 ± 1.3	1.257 ± 0.238	2.0 ± 3.9	1.423 ± 0.135
	2000	48 h	2.6 ± 2.1	1.610 ± 0.359	1.8 ± 1.6	1.576 ± 0.226
Positive control						
CPA	30	24 h	**16.2 ± 4.6***	1.217 ± 0.478	**17.6 ± 3.8***	1.109 ± 0.189
HCD mean ± SD	not specified	24 h	26 ± 14	1.31 ± 0.38	21 ± 11	1.37 ± 0.47
range	not specified		*8 - 81*	*0.603 - 2.623*	*4 - 67*	*0.143 - 2.534*

PCE: polychromatic erythrocytes; mPCE: micronucleated polychromatic erythrocytes; NCE: normochromatic erythrocytes
HCD: Historical control data, generated in the laboratory in 16 male and 16 female studies, conducted from 2002 - 2006
CPA: cyclophosphamide; * statistically significant when compared to solvent control

III. CONCLUSIONS

Based on the experimental findings [(Acetylamino)methyl]phosphonic acid did not induce micronuclei in bone marrow of male and female mice and is therefore considered negative for clastogenicity *in vivo*.

Assessment and conclusion by applicant:

In the present study, [(Acetylamino)methyl]phosphonic acid was negative for clastogenic and aneugenic effects in the bone marrow of male and female Crl:CD1 (ICR) mice *in vivo*.
The study was conducted under GLP conditions and in accordance with OECD guideline 474 (1997). Only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). Bone marrow toxicity, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, or systemic toxicity were not observed. However, the test was performed at limit dose levels in line with the current guideline. The deviations were considered to be of minor degree and do not compromise the validity of the study. The study is therefore considered valid.

Assessment and conclusion by RMS:

It is agreed with the applicant that, in this study, [(acetylamino)methyl]phosphonic acid was negative for clastogenic and aneugenic effects in the bone marrow of male and female Crl:CD1 (ICR) mice *in vivo*. The study is considered to be acceptable but with restrictions (reliable with restrictions) due to the deviation in the number of scored polychromatic erythrocytes. It should however be noted that bone marrow exposure was not shown that as there was no effect on the PCE/NCE ratio and no systemic exposure was observed.

The study was not available for the previous evaluation (RAR, 2015).

B.6.8.1.3. Studies with N-acetyl glyphosate

B.6.8.1.3.1. Absorption, Distribution, Metabolism and Excretion

Study 1

Data point:	CA 5.8.1/038
Report author	[redacted]
Report year	2004
Report title	Mass Balance, Metabolism, and Pharmacokinetics of [^{14}C]N-acetyl-glyphosate Following Administration of a Single Oral Dose to Rats
Report No	[redacted] 7535-100 Amended Report, [redacted] 56245A (Corteva)
Document No	NA
Guidelines followed in study	40 CFR 160, Guideline OPPTS 870.7485
Deviations from current test guideline	Yes, no full range ADME study, only males, only one dose
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 1 **Conclusion AGG:** The study is considered to be acceptable but with restrictions (reliable with restrictions)

Executive summary

The pharmacokinetics, absorption, elimination, and metabolism were studied in male rats following a single oral administration of [^{14}C]-N-acetyl-glyphosate at a nominal dose of 15 mg free acid equivalent/kg bw. Blood was collected from four rats per time point up to 72 hours post-dose. Excreta were collected from five animals at specified intervals through 7 days post-dose. Plasma, urine, and faeces were analysed for unchanged parent compound and metabolites.

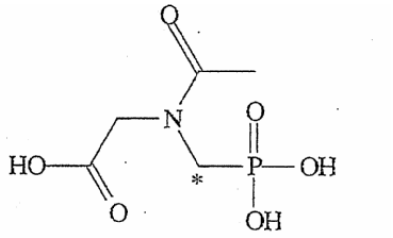
The mean total recovery of radioactivity was 95.5 %, with 66.1 % in urine, 26.4 % in faeces, 2.79 % in cage wash and wipe, and 0.23 % in residual carcass. More than 90 % of the total radioactivity was excreted within 48 hours. The mean maximum concentrations (Cmax) in blood and plasma were 2.93 and 5.31 µg equivalents/g 1 and 2 hours after application, respectively. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 hours, respectively. Comparison of blood and plasma values for area under the curve (AUC) indicated that [^{14}C]-N-acetyl-glyphosate distributed preferentially into plasma.

Unchanged [^{14}C]-N-acetyl-glyphosate recovered in urine and faeces represented over 99 % of the administered radioactivity. A metabolite, glyphosate, was detected in faeces and represented less than 1 % of the total radioactivity. Plasma radioactivity consisted entirely of unchanged [^{14}C]-N-acetyl-glyphosate.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:	N-acetyl-glyphosate
Identification:	N-acetyl-glyphosate, sodium salt
Description:	Not reported
Lot #:	123K5012 (PTRL West Log No. 1247W-001)
Purity:	84.3 % sodium salt (67.4 % based on free acid)

Stability of test compound:	Responsibility of sponsor
2. Radiolabelled test material:	[^{14}C]-N-acetyl-glyphosate
Identification:	N-acetylglyphosate-phosphonomethyl-^{14}C
Position of radiolabel:	
Lot #:	123K9416
Radiochemical purity:	99.181 % (confirmed by HPLC prior to dose preparation)
Specific activity:	8.0 mCi/mmol
Stability of test compound:	Stability was confirmed by pre- and post-dose HPLC analysis of radiochemical purity values
3. Analytical standard:	
Identification:	Glyphosate-phosphonomethyl - ^{14}C sodium salt
Lot No.	012K9430/31
Specific activity:	2.2 mCi/mmol
Radiochemical purity:	99.237 %
4. Vehicle:	Sterile water for injection
5. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crl:CD(SD)IGS BR)
Source:	[redacted]
Age:	10 weeks at dosing
Sex:	Males
Weight at dosing:	266 to 292 g at dosing
Acclimation period:	At least 4 days; all animals appeared clinically healthy throughout acclimation
In-life dates:	09 to 16 Mar 2004
Diet/Food:	Certified Rodent Diet #8728CM (Harlan Teklad, Inc.), *ad libitum*
Water:	Water, *ad libitum*
Housing:	<u>During acclimatisation:</u> Individual, suspended, stainless steel, wire mesh cages. <u>During test period:</u> Animals designated for collection of excreta were housed in individual Nalgene cages designed for the separation and collection of urine and faeces; Animals designated for pharmacokinetic analyses were housed in individual, suspended, stainless steel wire-mesh cages.
Environmental conditions:	Temperature: 22 ± 4 °C Humidity: 50 ± 20 % Air changes: not stated 12-hour light/dark cycle

B. STUDY DESIGN

Animal assignment and treatment

Administration

Group	Number of male rats	Route of dosing	Frequency of dosing	Dose level (mg/kg bw)	Dose Volume (mL/kg bw)	Radioactive dose (µCi/kg)	Sampling
1	5	Oral gavage	Single	15	5	100	Faeces, urine, cage wash, carcass
2	40*	Oral gavage	Single	15	5	100	Blood

*4 animals per blood sampling time point

Animals were allocated to the different dose groups randomized based on body weight. Animals were fasted overnight through approximately 4 hours after application of 15 mg [^{14}C]-N -acetyl-glyphosate/kg bw. The oral dose was administered via ball-tipped gavage needle.

Observation of animals

Cage-side observations

Animals were observed for mortality and signs of pain and distress twice daily. Cage side observations for general health and appearance were done once daily.

Body Weights

Animals were weighed at randomization and on the day of dose administration.

Animal assignment and treatment: Excretion/mass balance study (Group 1)

Each of five male rats received a single gavage administration of 15 mg [^{14}C]-N-acetyl-glyphosate/kg bw. Urine and faeces were collected at 0 - 6, 6 - 12, and 12 - 24 hour intervals and at 24-hour intervals through 168 hours. The weight of each sample was recorded. After the last excreta collections, cages were washed and wiped with a solution of methanol: 1 % trisodium phosphate (TSP) in water (50:50, v:v) and gauze pads. The cage wash samples and gauze were collected into separate plastic containers and the weight of each cage wash sample was recorded. Animals were sacrificed with an overdose of halothane anaesthesia and the residual carcass from each animal was retained.

Animal assignment and treatment: Pharmacokinetic study (Group 2)

Each male rat received a single gavage administration of 15 mg [^{14}C]-N-acetyl-glyphosate/kg bw. Four animals per time point were sacrificed via exsanguination (cardiac puncture) under halothane anaesthesia. Samples were collected pre-dose, and at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hours post-dose. At sacrifice blood was collected via syringe and needle into tubes containing K_3EDTA anticoagulant from each animal. Blood was aliquoted for radio-analysis and centrifuged to obtain plasma.

Sample Preparation and radio-analysis

All sample combustions were done in a Model 307 Sample Oxidizer (Packard Instrument Company) and the resulting $^{14}CO_2$ was trapped in a mixture of Perma Fluor and Carbo-Sorb. Ultima Gold XR scintillation cocktail was used for samples analysed directly. All samples were analysed for radioactivity in liquid scintillation counters (Packard Instrument Company) for at least 5 minutes or 100,000 counts. Each sample was homogenized before radio-analysis (unless the entire sample was used for analysis). All samples were analysed in duplicate if sample size allowed. If results from sample duplicates (calculated as ^{14}C dpm/g sample) differed by more than 10 % from the mean value, the sample was re-homogenized and re-analysed (if the sample size permitted). This specification was met for all sample aliquots that had radioactivity greater than 100 dpm. Scintillation counting data (cpm) were automatically corrected for counting efficiency using the external standardization technique and an instrument-stored quench curve generated from a series of sealed quenched standards.

Blood and Plasma

Duplicate weighed aliquots of blood and plasma were analysed by LSC.

Urine and Cage Wash
Duplicate weighed aliquots of urine were analysed directly by LSC. If no urine sample was visible, a sufficient amount of water was added to thoroughly rinse the container, the sample was mixed by shaking, and duplicate aliquots were taken and analysed by LSC.

Faeces
A sufficient amount of methanol:water (50:50, v:v) was added to facilitate homogenization. Duplicate weighed aliquots were combusted and analysed by LSC.

Cage Wipe
A sufficient amount of methanol:water (50:50, v:v) was added to cover each sample. Samples were allowed to extract, mixed by gentle shaking, and duplicate weighed aliquots were analysed by LSC.

Carcass (residual)
Each sample was cut into pieces, which were frozen in liquid nitrogen and homogenized in a Wiley Mill that had been precooled with dry ice. Additional dry ice was ground to clear any remaining sample from the mill. After the dry ice had sublimed, the homogenate was digested in 1N sodium hydroxide. Duplicate weighed aliquots were taken and hydrogen peroxide was added in a dropwise fashion. After any foaming had dissipated, Ultima Gold XR scintillation cocktail was added, the aliquots were allowed to sit overnight and analysed by LSC.

Characterisation of Metabolites
Group 1 urine samples collected at 0 - 6, 6 - 12, 12 - 24, 24 - 48, and 48 - 72 hours post-dose were pooled across animals by time point. (Urine collected from Animal No. Cl6498 was excluded from the pool due to suspected cross-contamination of radioactivity between urine and faeces for this animal). Each pool was analysed by LSC. The pools were microfuged, and the supernatants were analysed by LSC and HPLC to determine the metabolic profile. The HPLC eluents for each pooled sample were also analysed by LSC.

Group 1 faeces samples collected from 6 - 12, 12 - 24, and 24 - 48 hours post-dose were pooled across animals by time point. (Faeces collected from Animal No. Cl6498 were not included in the pools; however, samples collected from this animal at 12 - 24 and 24 - 48 hours post-dose were analysed separately.) In general, aliquots from each pool or sample were oxidized and analysed by LSC. Approximately 1 to 5 g of each faeces pool or sample was extracted twice with approximately 10 mL of methanol. The samples were sonicated, shaken, and centrifuged. The supernatants were combined and analysed by LSC. The extraction solvent was evaporated under nitrogen. The remaining solids were then extracted twice with approximately 10 mL of mobile phase A (50 mM potassium phosphate, monobasic, pH 2:methanol 96:4, v:v). Samples were sonicated, vortex mixed, and centrifuged. The resulting mobile phase A supernatants were combined and analysed by LSC and HPLC. For two samples, the 6 to 12-hour pool and the 24-hour single sample, the methanol residues and the mobile phase A extracts were combined and the solvent was evaporated under nitrogen. The samples were reconstituted in mobile phase A:methanol (9:1, v:v), and microfuged. The resulting supernatants were analysed by HPLC to determine the metabolite profile. The HPLC column eluents for each pooled sample were also analysed by LSC. Following extraction and centrifugation, remaining solids were allowed to air dry, weighed, combusted, and analysed by LSC to determine the total extraction recovery.

Group 2 plasma samples collected at 0.5, 1, 2, 4, 8, 12, and 24 hours post-dose were pooled across animals by time point and the radioactivity in each pool was analysed by LSC. Approximately 3 to 6 mL of each plasma pool was extracted twice with approximately 3 times the volume of methanol. Samples were vortex mixed, sonicated, and centrifuged. The extracts were combined, analysed by LSC, and evaporated to near dryness. Plasma supernatants from the 8-, 12-, and 24-hour pools were further cleaned (using C18 solid phase extraction cartridge and eluting with water and methanol) then again reduced to dryness. Each sample was reconstituted in an appropriate volume of mobile phase A (50 mM potassium phosphate, monobasic, pH 2:methanol 96:4, v:v) and methanol (6:4 v:v). The samples were microfuged and each supernatant was analysed by HPLC to determine the metabolite profile. The post-extraction residues were dried, weighed, combusted, and analysed by LSC to determine the total extraction recovery.

Dose Preparation, Verification and Stability
The oral dose of [^{14}C]-N -acetyl-glyphosate was formulated as a solution in sterile water for injection at a nominal concentration of 3 mg/mL. The day before dose administration, 1.80 mL (containing 47.5 mg free acid)

of [^{14}C]-N-acetyl-glyphosate and 331.8 mg N-acetyl-glyphosate salt were transferred into a serum vial and combined with 88.2 mL of the dose vehicle. Duplicate weighed aliquots were taken from the dose formulation prior to and following dose administration, and were analysed by liquid scintillation counting (LSC) to determine the concentration of radioactivity and homogeneity. Stability was demonstrated by analysing pre-dose and post-dose aliquots by HPLC.

LC/MS and LC/MS/MS Analyses
Selected urine samples, and extracts of faeces and plasma were analysed using liquid chromatography mass spectrometry (LC/MS) ionised via negative electro spray ionisation. Based on the full scan LC/MS data, a number of LC/MS/MS analyses were performed for structural elucidation. The LC/MS /MS analyses used the same instrumentation and conditions as for the negative ion full-scan LC/MS with minor modifications.

Data Analyses
Statistical analyses were limited to simple expressions of variation, such as mean and standard deviation. Dose tables were compiled with mean and standard deviation values calculated using Excel, Version 8.0e (Microsoft Corporation). Data tables were generated by Debra, Version 5.2a (LabLogic Systems Ltd., Sheffield, UK). Debra is an automated and validated data capture and management system for data collection in absorption, distribution, and excretion studies using radiolabeled test article. Debra captures data from balances and scintillation counters. The maximum concentration (Cmax) in blood and plasma and the time to reach maximum concentration (Tmax) were obtained by visual inspection of the raw data. Pharmacokinetic parameters calculated included half-life ($t_{1/2}$), area under the concentration-time curve from time 0 to the last measurable time point (AUC_{0-t}), and area under the concentration-time curve from 0 to infinity ($AUC_{0-\infty}$). Pharmacokinetic parameters were calculated by using WinNonlin Professional Edition (Pharsight Corporation, Mountain View, CA).

Data evaluation
If results of sample analysis are below twice background, Debra reports the result as less than the limit of quantitation (BLQ). Values of BLQ are included as 0 in calculations of mean and variance. The concentration associated with the limit of quantitation is calculated as follows:
Limit of Quantitation (µg equivalents/g) = 2× Background (dpm)+Aliquot weight (g) f /Dose specific activity (dpm/µg)

The percent of administered dose associated with the limit of quantitation is calculated as follows:
Limit of quantitation (% of dose) = Limit of Quantitation (µg equivalents/g) × Sample weight (g) × 100/ µg administered

II. RESULTS AND DISCUSSION

A. TEST ARTICLE AND DOSE FORMULATION ANALYSIS

Radiochemical purity and Stability
The HPLC analyses showed the radiochemical purity of [^{14}C]-N-acetyl-glyphosate to be 99.8 % prior to dosing. The mean radiochemical purity values obtained from HPLC analysis of pre-dose and post-dose aliquots from the dose solution were 99.9 % and 99.2 %, respectively, confirming stability of the test article under the conditions of the study. Representative stability chromatograms were presented.

Concentration and Homogeneity
The LSC analysis results indicated the dose solution was homogenous during the dosing period.

Specific Activity
The specific activity of the [^{14}C]-N-acetyl-glyphosate as provided by the sponsor was 37.9 µCi/mg. The mean specific activity of [^{14}C]-N-acetyl-glyphosate in the dose solution was determined to be 7.05 µCi/mg.

B. DOSE ADMINISTRATION
The volume of radiolabelled dose formulation to be administered to each animal by oral gavage was calculated based on the body weight taken on the day of dose administration. The actual amount administered was determined by weighing the dose syringe before and after dose administration. All animals were fasted overnight through approximately 4 hours post-dose.

Table 6.8.1.3.1.1-37: Mean body weights and radioactivity doses administered to male rats dosed orally with [^{14}C]-N-acetyl-glyphosate at a target dose of 15 mg/kg

Group	Number of male rats		Body weight (g)	Dose weight (g)	Actual dose administered				
					(dpm)	(mg/animal)	(mg/kg)	(µCi/animal)	(µCi/kg)
1	**5**	**Mean**	280	1.39	66457821	4.25	15.1	30.0	107
		SD	8	0.05	2375031	0.15	0.2	1.1	2
2	**40**	**Mean**	277	1.38	66246716	4.24	15.3	29.8	108
		SD	8	0.04	2114489	0.14	0.2	1.0	1

SD: Standard deviation. Dose concentration was 4.79 x 10^7 dmp/g. Dose specific activity was 7.05 µCi/mg.

C. CAGE-SIDE OBSERVATIONS

All animals appeared healthy and exhibited no overt signs of toxicity throughout the study.

D. EXCRETION AND MASS BALANCE

The mean total recovery of radioactivity from the five animals in Group 1 was 98.0 % with 57.2 % in urine, 37.6 % in faeces, 2.91 % in cage wash and wipe, and 0.26 % in residual carcass. More than 90 % of the total radioactivity was eliminated within 48 hours, with renal excretion being the primary elimination route. For animal No. C16498, 82.4 % of the administered radioactivity was detected in faeces, indicating probable urine contamination of the faeces. Excluding this animal, mean total recovery was 95.5 %, with 66.1 % in urine, 26.4 % in faeces, 0.23 % in carcass, and 2.79 % in cage wash/wipe.

Table 6.8.1.3.1.1-38: Percent of radioactive dose in urine, faeces, cage wash, cage wipe and carcass at specified intervals after a single oral administration of [^{14}C]-N-acetyl-glyphosate to male rats (n=5) at 15 mg/kg bw

Collection interval (hours)	% of applied radioactive dose			
	Mean	SD	Mean[a]	SD[a]
Urine				
0 - 6	40.5	17.5	47.7	8.0
6 - 12	11.8	7.8	13.6	7.7
12 - 24	2.13	0.70	2.06	0.79
24 - 48	1.35	0.66	1.37	0.77
48 - 72	0.48	0.35	0.43	0.39
72 - 96	0.29	0.19	0.26	0.21
96 - 120	0.42	0.37	0.46	0.42
120 - 144	0.17	0.09	0.18	0.10
144 - 168	0.12	0.10	0.13	0.12
Subtotal	57.2	23.5	66.1	14.3
Faeces				
0 - 6	0.00	0.00	0.00	0.00
6 - 12	5.56	7.65	6.95	8.07
12 - 24	24.2	18.7	16.2	6.8
24 - 48	7.20	10.3	2.68	2.1
48 - 72	0.22	0.18	0.17	0.16
72 - 96	0.17	0.18	0.11	0.14
96 - 120	0.16	0.12	0.16	0.14
120 - 144	0.05	0.04	0.05	0.04
144 - 168	0.03	0.01	0.03	0.02
Subtotal	37.6	27.7	26.4	13.5
Cage wash, cage wipes and carcass				
168 (cage wash)	0.61	0.31	0.69	0.28
168 (cage wipe)	2.30	2.30	2.10	2.61
168 (carcass)	0.26	0.07	0.23	0.04

Table 6.8.1.3.1.1-38: Percent of radioactive dose in urine, faeces, cage wash, cage wipe and carcass at specified intervals after a single oral administration of [^{14}C]-N-acetyl-glyphosate to male rats (n=5) at 15 mg/kg bw

Collection interval (hours)	% of applied radioactive dose			
	Mean	SD	Mean[a]	SD[a]
Total	**98.0**	**5.7**	**95.5**	**1.2**

SD: Standard deviation. a: Calculated excluding values for Animal No. C16498, which had suspected urine contamination of faeces

E.PHARMACOKINETICS

Following a single oral administration, the mean maximum concentrations (C_{max}) in blood and plasma were 2.93 and 5.31 µg equivalents/g after 1 and 2 hours, respectively. The values for half-life ($t_{1/2}$), and area under the curve (AUC_{0-t}, and $AUC_{0-\infty}$) were 20.1 hours, 12.0 µg equivalents-hour/g, and 12.1 µg equivalents- hour/g in blood and 15.6 hours, 20.7 µg equivalents-hour/g and 20.8 µg equivalents-hour/g in plasma.

Table 6.8.1.3.1.1-39: Concentrations of radioactivity in blood and plasma at specified times after a single oral administration of [^{14}C]N-acetyl-glyphosate to male rats at 15 mg/kg bw

Collection time point (hours)	Blood		Plasma	
	µg Eq [^{14}C]N-acetyl-glyphosate/g		µg Eq [^{14}C]N-acetyl-glyphosate/g	
	Mean	SD	Mean	SD
0.5	2.17	0.65	3.89	1.04
1	2.93	1.25	5.29	2.24
2	2.84	0.87	5.31	1.60
4	0.983	0.164	1.79	0.29
8	0.0973	0.0325	0.142	0.061
12	0.0442	0.0041	0.0493	0.0076
24	0.0243	0.0012	0.0166	0.0029
48	0.00994	0.00669	0.00895	0.00090
72	0.00548	0.00635	BLQ	BLQ

SD: Standard deviation; Eq: Equivalents. BLQ = below limit of quantitation. Four rats were sacrificed per sampling time

Table 6.8.1.3.1.1-40: Pharmacokinetic parameters for radioactivity in blood and plasma collected from male rats after a single oral administration of [^{14}C]-N-acetyl-glyphosate at 15 mg/kg bw

Matrix	T_{max} (hours)	C_{max} (µg eq/g)	$t_{1/2}$ (hours)	$AUC_{(0-t)}$ (hours µg Eq/g)	$AUC_{(0-\infty)}$ (hours µg Eq/g)
Blood	1	2.93	20.1	12.0	12.1
Plasma	2	5.31	15.6	20.7	20.8

Eq: Equivalents

F. METABOLITE CHARACTERISATION

Nearly 100 % of the radioactivity was recovered in pooled urine after micro-centrifugation for HPLC analysis. Total extraction recoveries for pooled faeces ranged from 93.2 to 106 %, with 92.9 to 101 % extracted and 0.15 to 5.75 % remaining in the post extraction solid. The total extraction recoveries for pooled plasma collected from 0.5 to 8 hours post-dose ranged from 97.1 to 105 %, with 88.0 to 103 % extracted and 1.35 to 9.05 % remaining in the post extraction solid. Due to low levels of radioactivity in the samples collected at 12 and 24 after dosing, recoveries were low in these samples. Extracted radioactivity from selected samples was analysed by using HPLC with radioactivity flow detection and LC/MS/MS.

One radiolabelled component was detected by HPLC analysis of urine and extracts of faeces and plasma. This component had retention times similar to the [^{14}C]-N-acetyl-glyphosate reference standard when the samples and the standard were co-injected. LC/MS/MS analysis of this component in urine, plasma, and faeces provided

molecular ion and production spectra that were virtually identical to that of the [^{14}C]-N-acetyl-glyphosate reference standard.

Urine

The HPLC profile in urine showed only unchanged [^{14}C]-N-acetyl-glyphosate. A total of 65.2 % of the administered [^{14}C]-N-acetyl-glyphosate was excreted in urine.

Faeces

The HPLC profile in faeces showed mostly unchanged [^{14}C]-N-acetyl-glyphosate, with trace amounts of a metabolite. This metabolite was identified as glyphosate by co-chromatography of a faeces extract with the [^{14}C]-glyphosate reference standard; the metabolite was not confirmed by LC/MS analysis, due to the low levels of radioactivity in faeces samples.

Plasma

The HPLC profile for plasma showed only unchanged [^{14}C]-N-acetyl-glyphosate in samples collected from 0.5 to 12 hours post-dose.

Table 6.8.1.3.1.1-41: Detected metabolites in pooled samples following a single oral administration of [^{14}C]-N-acetyl-glyphosate at 15 mg/kg bw to male rats (n=5)

Pooled sample type	Proposed identification	Retention time (minutes)	Percent of Sample Radioactivity					
			0 - 6h	6 - 12h	12 - 24h	24 - 48h	48 - 72h	
Urine	N-acetyl-glyphosate	9.2 – 9.6	99.8	100	100	100	100	
Faeces	Glyphosate	5.4-6.0	No sample	ND	0.47 (0.45)	ND (0.75)	No sample	
	N-acetyl-glyphosate	8.1-8.7		100	99.5 (99.6)	100 (99.3)		
			Percent of radioactive Dose					
			0 - 6h	6 - 12h	12 - 24h	24 - 48h	48 - 72h	
Urine	N-acetyl-glyphosate	9.2 – 9.6	47.7	13.6	2.06	1.37	0.43	
Faeces	Glyphosate	5.4-6.0	No sample	ND	0.07 (0.24)	ND (0.19)	No sample	
	N-acetyl-glyphosate	8.1-8.7		13.9	15.5 (52.3)	2.59 (24.6)		
			Percent of Sample Radioactivity					
			0.5h	1h	2h	4h	8h	12h
Plasma	N-acetyl-glyphosate	9.3 – 9.9	100	100	100	100	100	100
			Concentration (µg Eq [^{14}C]-N-acetyl-glyphosate/g)					
			0.5h	1h	2h	4h	8h	12h
	N-acetyl-glyphosate	9.3 – 9.9	4.13	5.40	5.25	1.86	0.126	0.287

ND = not detected. Values in brackets () were detected in the individual sample from Animal No. C16498

III. CONCLUSIONS

Following a single oral administration of [^{14}C]-N-acetyl-glyphosate to male rats, approximately 66 % of the total radioactivity was excreted in urine and approximately 26 % in faeces. Over 90 % of the radioactivity was excreted within 48 hours post-dose. Minimal metabolism occurred, with unchanged [^{14}C]-N-acetyl-glyphosate representing more than 99 % of the total administered radioactivity detected in urine and faeces. Trace amounts of [^{14}C]-glyphosate, representing less than 1 % of the total radioactivity, were detected in faeces. [^{14}C]-N-acetyl-glyphosate was the only circulating radioactive component in plasma, and was eliminated from plasma with a half-life of 15.6 hours.

Assessment and conclusion by applicant:

A single oral dose of [^{14}C]-N-acetyl-glyphosate at 15 mg free acid equivalent/kg body weight was administered to five male Sprague-Dawley rats. Urine and faeces were collected at different time points. Upon sacrifice the cage was and carcasses were analysed for radioactivity. The mean total recovery of radioactivity after a collection period of 7 days was 95.5 % with 66.1 % in urine, 26.4 % in faeces, 2.79 % in cage wash and wipe, and 0.23 % in residual carcass.

Pharmacokinetics
Blood was collected over a sufficient time period as only trace amounts were detectable at 72 hours post-dose. [^{14}C]-N-acetyl-glyphosate was the only circulating radioactive component in plasma, and was eliminated from plasma with a half-life of 15.6 hours. The mean maximum concentrations (Cmax) in blood and plasma were 2.93 and 5.31 µg equivalents/g at 1 and 2 hours post-dose, respectively. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 hours, respectively. Comparison of blood and plasma values for area under the curve (AUC) indicated that [^{14}C]-N-acetyl-glyphosate distributed preferentially into plasma.
The limit of quantitation was not explicitly stated in the report, but was <0.01 µg Equivalents [^{14}C]-N-acetyI-glyphosate/g for blood and plasma.

Absorption
N-acetyl-glyphosate is quickly absorbed after oral application as indicated by the urinary excretion. 47.7 % of the applied radioactivity was excreted within 6 h after application. 63.4 % of the applied radioactivity was excreted within 24 h (excluding cage wash). This supported by Tmax values being 1 and 2 hours in blood and plasma, respectively.

Elimination
Approximately 66 % of the total radioactivity was excreted in urine and approximately 26 % in faeces. Over 90 % of the radioactivity was eliminated within 48 hours post-dose.

Metabolism
No metabolites were detected in plasma and urine. Plasma and urine radioactivity consisted entirely of unchanged [^{14}C]-N-acetyl-glyphosate. Unchanged [^{14}C]-N-acetyl-glyphosate recovered in urine and faeces represented over 99 % of the administered radioactivity. A metabolite found at less than 0.25 % of the administered radioactivity in faeces was glyphosate, which was formed by N-deacetylation.

Conclusion:
The study mainly focused on metabolism, elimination and pharmacokinetics of N-acetyl-glyphosate after single oral administration to male rats. Methods and results are acceptable and can be used as reliable supplementary data.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. Due to the limitations the study is considered acceptable buth with restrictions. At 15 mg/kg bw N-acetyl-glyphosate shows rapid elimination and minimal metabolism in male rats. As no information was provided for females no conclusion can be drawn on possible sex differences in kinetic properties.

B.6.8.1.3.2. Acute oral toxicity

Study 1

1. Information on the study

Data point	CA 5.8.1/039
Report author	[redacted]
Report year	2004
Report title	Acute Oral Toxicity Study in Rats with N-Acetyl-Glyphosate, Sodium Salt (Acute Toxic Class Method)
Report No	7535-103

Document No	25930-0-804
Guidelines followed in study	US EPA 870.1100 – Acute Oral Toxicity (1998); OECD 423 (2001)
Deviations from current test guideline (OECD 423, 2001)	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 1) **Conclusion AGG:** The study is considered to be acceptable.

Executive summary
The toxicity of the test article, N-acetyl-glyphosate (as the free acid), was evaluated following a single oral dose to the rat. The test article was suspended in cell culture grade water and administered by oral gavage at a dose level of 5000 mg/kg bw to five rats/sex. The rats were observed for mortality, body weight effects and clinical signs for 15 days after dosing. All animals were subjected to an abbreviated gross necropsy examination of the external features of the carcass, external body orifices, the abdominal, thoracic and cranial cavities and organs/tissues.
Mortality was observed in one female at the 4-hour post-dose observation interval and in one male and one female during the a.m. check on Day 2. All the remaining males and females survived until the scheduled sacrifice. Clinical observations observed in the males included hypoactivity, irregular respiration, liquid or soft feces, light-brown perineal staining, brown nasal crust and/or squinted eyes. Clinical observations observed in the females included slight hypoactivity, irregular respiration, liquid or soft feces and/or light-brown perineal staining. All clinical signs of toxicity were resolved in all surviving animals by Day 3.
All surviving animals gained weight from initiation of dosing to study termination. At necropsy, findings were noted in the one male and two females that were found dead prior to the terminal sacrifice, however no abnormal findings were noted in the remaining animals at necropsy. Findings in one male and one female involved the lungs (mottled or discolored bright-red), liver (discolored black), stomach (soft and/or with yellow fluid), abdominal cavity (fluid/clear fluid), and duodenum, jejunum and ileum (fluid). Finding in the second male involved the stomach (gel-like clear liquid and red walls), abdominal cavity (reddish-liquid), duodenum, jejunum and ileum (fluid).
Under the conditions of this study, the oral LD_{50} of the test article is greater than 5000 mg/kg bw. The mortality rate was 20% in the males and 40% in the females dosed at 5000 mg/kg.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	N-acetyl-glyphosate, sodium salt
Description:	White powder
Lot/Batch #:	123K5012
Purity:	84.3% sodium salt; 67.4% free acid
Stability of test compound:	The test substance appeared to be stable under the conditions of the study (sponsor's responsibility). Expiry date: 2006-02-10.

2. Vehicle and/ or positive control: Cell culture grade water (Bio Whittaker/Cambrex; Lot No. 01102570; Expiration Date: 2005-08-04) / none

3. Test animals:

Species:	Rat
Strain:	Crl:CD®(SD)IGS BR
Source:	[redacted]
Age:	8 – 12 weeks
Sex:	Male and female

Weight at dosing:	♂ 223 – 247 g; ♀ 231 – 242 g
Acclimation period:	5 days
Diet/Food:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Individually in sanitary, stainless-steel, screen-bottomed, hanging, wire cages
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 50 ± 20 % Air changes: ≥10 air changes / hour 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2004-04-01 to 2004-04-16

Animal assignment and treatment:
A limit test using five animals/sex at a dose level of 5000 mg/kg bw was conducted. The animals were fasted overnight (17 to 20 hours before test material administration) and for approximately 4 hours after dosing. Dose calculations were adjusted by a factor of 67.4% to obtain a dose of the free acid. On the day of dosing the test material was suspended in the vehicle, forming an opaque, viscous, white, homogeneous suspension. Individual doses were calculated based on each animal's fasted body weight taken just before test material administration and a dose volume of 10 mL/kg bw.
The test article was administered orally via a gavage needle attached to a disposable syringe. An assigned syringe and oral gavage needle was used for each dosing group. Prior to dose administration to each animal, the gavage needle was wiped with paper towels.

Table B.6.8.1.3.2.1-1: Acute Oral Toxicity Study in Rats with N-Acetyl-Glyphosate, Sodium Salt (Acute Toxic Class Method) ([redacted], 2004): Study design and mortality

Test group	Dose level [mg/kg bw]	Mortality / number of animals	
		Males	Females
N-acetyl-glyphosate, sodium salt	5000	1/5	2/5

Analysis of the test substance
The analysis of the test substance was in the responsibility of the sponsor.

Mortality
The animals were observed for general health and mortality at least once daily during acclimation and twice daily (at least 4 hours apart) for the duration of the study.

Clinical observations
Observations were performed for clinical signs of toxicity pre-dose on the day of dosing (Day 1), continuously for the first hour post-dose and at approximately 4 and 6 hours post-dose. Daily observations were performed thereafter until the day prior to sacrifice.

Body weight
The animals were weighed pre-dose on the day of dosing (Day 1; fasted), on Days 8 and 15, and at termination (Day 16; fasted).

Sacrifice and pathology
After 15 days of observation, all surviving animals were food fasted overnight, weighed (Day 16) and anesthetised with an appropriate barbiturate.

Necropsy
All animals were subjected to an abbreviated gross necropsy examination of the external features of the carcass,

external body orifices, the abdominal / thoracic / cranial cavities and organs / tissues. Any abnormalities were recorded. After necropsy, the animals were discarded; no tissues were saved.

Statistics
Other than the calculation of the LD_{50} and the descriptive statistics of the body weights (when applicable) no statistical analyses were required.

II. RESULTS AND DISCUSSION

A. MORTALITY
Mortality was observed in one female at the 4 hour post-dose observation interval and in one male and one female during the a m. check on Day 2. All the remaining males and females survived until the scheduled sacrifice. Please refer to the table above.

B. CLINICAL OBSERVATIONS
Clinical observations observed in the males included slight and distinct hypoactivity, irregular respiration, liquid or soft faeces, brown nasal crust, light-brown perineal staining and/or squinted eyes. Clinical observations observed in the females included slight hypoactivity, irregular respiration, liquid or soft faeces and/or light-brown perineal staining. All clinical signs of toxicity were resolved in all surviving animals by Day 3.

C. BODY WEIGHT
All surviving animals gained weight from initiation of dosing to study termination.

D. NECROPSY
Findings were noted in the one male and two females that were found dead prior to the terminal sacrifice, however no abnormal findings were noted in the remaining animals at necropsy. Findings in one male and one female involved the lungs (mottled or discoloured bright-red), liver (discoloured black), stomach (soft and/or with yellow fluid), abdominal cavity (fluid/clear fluid), and duodenum, jejunum and ileum (fluid). Finding in the second male involved the stomach (gel-like clear liquid and red walls), abdominal cavity (reddish-liquid), and duodenum, jejunum and ileum (fluid).

Table B.6.8.1.3.2.1-2: Acute Oral Toxicity Study in Rats with N-Acetyl-Glyphosate, Sodium Salt (Acute Toxic Class Method) ([redacted], 2004): Necropsy findings

Animal number	Sex	Dose level [mg/kg bw]	Day of death	Necropsy Observation
5045	Male	5000	Terminal	No abnormal findings
5046	Male		Terminal	No abnormal findings
5047	Male		Terminal	No abnormal findings
5048	Male		Day 2	Lungs – mottled Liver – discoloured black Stomach – soft Abdominal cavity – clear fluid Duodenum, jejunum, ileum – fluid
5049	Male		Terminal	No abnormal findings
5050	Female		Terminal	No abnormal findings
5051	Female		Day 2	Lungs – discoloured bright-red Liver – discoloured black Stomach – soft with yellow fluid Abdominal cavity – fluid Duodenum, jejunum, ileum – fluid
5052	Female		Day 1	Stomach – gel-like clear liquid, red walls Abdominal cavity – reddish liquid Duodenum, jejunum, ileum – fluid
5053	Female		Terminal	No abnormal findings
5054	Female		Terminal	No abnormal findings

III. CONCLUSIONS
Under the conditions of this study, the oral LD_{50} of the test article is greater than 5000 mg/kg bw. The mortality rate was 20 % in the males and 40 % in the females dosed at 5000 mg/kg bw. The test material, N-acetyl-

glyphosate, sodium salt was toxic when administered once by oral gavage at a dose level of 5000 mg/kg bw (as the free acid) in male and female rats under the conditions of this assay.

Assessment and conclusion by applicant:
This study is considered to be valid as it was conducted under GLP and no deviations from the recent guideline could be identified.
Under the conditions of this study, the oral LD_{50} of N-acetyl-glyphosate, sodium salt is greater than 5000 mg/kg bw.

Assessment and conclusion by RMS:
It is agreed with the applicant. The acute oral LD_{50} of N-acetyl glyphosate in male and female rats was determined to be > 5000 mg/kg bw in this study. All animals showed clinical signs in the course of the study. One male and two females died before study end and findings were noted in these animals during necroscopy. The study was not available for the previous assessment (RAR, 2015).

B.6.8.1.3.3. Sort-term toxicity

Study 1

Data point	CA 5.8.1/040
Report author	[redacted] supplement author)
Report year	2007
Report title	IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats
Report No	[redacted]-19008
Document No	NA
Guidelines followed in study	OPPTS 870.3100 (1998); OECD Guideline 408 (1998); Method B.26, Directive 2001/59/EC (2001); MAFF 2-1-9 Notification 12 Nousan 8147 and Notification 13 Seisan 1739 (2000 and 2001)
Deviations from current test guideline (OECD 408, 2018)	Clinical chemistry was performed without determining LDL, HDL, T3, T4 and TSH; organ weights were determined without prostate (+ seminal vesicles and coagulating glands), thyroid and pituitary gland; histopathology was performed without cervix, coagulating glands, gall bladder and male mammary glands.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

Executive summary
The objective of this study was to assess the potential sub-chronic toxicity of IN-MCX20 in rats. Five groups of young adult male and female Crl:CD(SD) rats (10/sex/group) were administered diets that contained 0, 180, 900, 4500 or 18000 ppm IN-MCX20 (equivalent to 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day for males and 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day for females) for approximately 90 days (95 days in males and 96 days in females). Samples of the diets were analysed to assess homogeneity, stability, and concentration of the test substance in the diet. Body weights, food consumption and detailed clinical observations were evaluated weekly. Ophthalmological and neurobehavioral (abbreviated functional observational battery, grip strength, motor activity) evaluations were performed on all rats prior to the start of dietary exposure and near the end of the exposure period. Clinical pathology endpoints (haematology, coagulation, clinical chemistry and urinalysis with metabolic analysis of IN-MCX20 (N-acetyl glyphosate) and its possible metabolites, IN-B2856 (glyphosate) and

IN-EY252 (N-acetyl AMPA)) were evaluated at the end of the exposure period. After approximately 90 days of dietary exposure, the surviving rats were sacrificed and given a gross and microscopic pathological examination.

Analysis of the diets demonstrated that IN-MCX20 was present at the targeted concentrations and was stable under relevant storage conditions. The homogeneity of the test substance in the initial diet preparations, in particular at the 180 ppm concentration, indicated a coefficient of variance (CV) of >10 %. However, most individual diet samples were within the targeted concentration range, and analysis of subsequent diet preparations supported adequate homogeneity. Therefore, it was concluded that the rats were exposed to the targeted concentrations of test substance.

The overall mean daily intake of IN-MCX20 in the 0, 180, 900, 4500 or 18000 ppm groups was 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day, respectively, for male rats and 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day, respectively, for female rats.

No adverse test substance-related effects on any body weight or nutritional parameters were observed. Statistically significantly lower overall mean body weight gain (86 % of control) was observed in 18000 ppm males but was not considered adverse as it was not associated with a statistically significant difference in mean final body weight or in overall mean food consumption or food efficiency. However, the RMS disagrees and considers the 14% decrease in body weight gain in males dosed at 18000 ppm as adverse and treatment-related. This decrease was associated with an 8% decrease in final body weight compared to controls.

No test substance-related deaths occurred and no clinical, ophthalmological or neurobehavioral observations were attributed to exposure to the test substance. There were no adverse effects on clinical pathology parameters, organ weights, gross pathology, or microscopic pathology in male or female rats.

In contrast to the conclusion by the applicant, the RMS considers the statistically significant decrease in body weight gain (day 0-91) of 14% compared with controls in males dosed at 18000 ppm as treatment-related and adverse. This effect was associated with an 8% decrease in final body weight compared to controls. This dose level of 18000 ppm (1157 mg/kg bw/day) in males is considered the LOEAL and the NOAEL is 4500 ppm (283 mg/kg bw/day).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Disodium N-acetyl-N-(phosphonomethyl)glycine
Identification:	IN-MCX20
Description:	White solid
Lot/Batch #:	27419
Purity:	63 %
Stability of test compound:	The test substance appeared to be stable under the conditions of the study and the study was completed prior to the expiration date on the Certificate of Analysis. No evidence of instability, such as a change in colour or physical state, was observed.
2. Vehicle and/ or positive control:	Diet / none
3. Test animals:	
Species:	Rat
Strain:	Crl:CD(SD)
Source:	[redacted]
Age:	7 weeks
Sex:	Male and female

Weight at dosing:	♂ 235.1 – 236.5 g; ♀ 173.8 – 175.9 g
Acclimation period:	6 days
Diet/Food:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel, wire-mesh cages suspended above cage boards
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 30 – 70 % Air changes: Not reported 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2007-05-18 to 2007-02-16

Animal assignment and treatment:
The test substance, IN-MCX20, was administered on a continuous basis in the basal diet for 95 or 96 days to 4 groups of (Crl:CD(SD)) rats. Dose levels were 180, 900, 4500 and 18000 ppm (equivalent to 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day for males and 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day for females). A concurrent control group received the basal diet on a comparable regimen. Each group consisted of 10 animals per sex.

Table 6.8.1.3.3.1-42: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Study design

Test group	Dietary concentration [ppm][1]	Mean daily intake mg/kg bw/day	Number of animals	
			Males	Females
Control	0	♂: 0; ♀: 0	10	10
Low	180	♂: 11.3; ♀: 13.9	10	10
Intermediate low	900	♂: 55.7; ♀: 67.8	10	10
Intermediate high	4500	♂: 283; ♀: 360	10	10
High	18000	♂: 1157; ♀: 1461	10	10

[1] Weight/weight concentration of test substance (adjusted for sponsor-supplied 63% purity of active ingredient).

Analysis of the test substance
IN-MCX20 was initially hand mixed with 500 grams of chow. Subsequently, this pre-mix was added to the diet and thoroughly mixed for a period of time that, by experience, was considered adequate to ensure homogeneous distribution in the diet. The manufacturer and production code of the feed was recorded in the study records. Diets were prepared weekly and refrigerated until used.
Duplicate samples (concentration and homogeneity verification) of all dietary concentrations were collected at the initial diet preparation and analysed to verify the concentration of IN-MCX20 in the diets. Duplicate samples were taken from the lowest and highest dietary concentration of the initial diet preparation and analysed to verify the stability of IN-MCX20 in the diets. The stability of IN-MCX20 in feed was demonstrated by comparing samples stored at room temperature or refrigerated with the mean measured values of the concentration (middle homogeneity) verification samples. Because of difficulties encountered with development of an analytical method, insufficient diet samples collected at study start were available to verify homogeneity in the diet. The analytical results of the middle homogeneity samples from this time point were used to support concentration at study start. Additional diet samples were collected from the top, middle, and bottom of the diet mixer of all dietary concentrations during the middle of the study and evaluated to confirm homogeneity of mixing, as well as to verify concentration at this time point. Toward the end of the study, additional samples were taken to verify concentration.

Mortality
Cage-site examinations to detect moribund or dead rats were conducted at least twice daily throughout the study.

Clinical observations
Cage-site examinations to detect abnormal behaviour and/or appearance among rats were conducted at least twice daily throughout the study. Abnormal behaviour/appearance was recorded by exception.

An additional cage-site evaluation was conducted daily, except on the days when detailed clinical observations were conducted, at approximately the same time (± 2 hours). Acute clinical signs of systemic toxicity were recorded.
At every weighing, each rat was individually handled and examined for abnormal behavior and appearance. Detailed clinical observations in a standardised arena were also evaluated on all rats. The detailed clinical observations included (but were not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection and unusual respiratory pattern), changes in gait, posture, response to handling, presence of clonic, tonic, stereotypical or bizarre behavior. Any abnormal clinical signs noted were recorded.

Body weight
All rats were weighed once per week.

Food consumption and test substance intake
The amount of food consumed by each rat over each weighing interval was determined throughout the study. From these determinations and body weight data, mean daily food consumption, mean food efficiency, and mean daily intake of IN-MCX20 were calculated.

Abbreviated Functional Observational Battery (FOB)
Sensory and motor function assessments were conducted by evaluating grip strength, responses to approach/touch, sharp auditory stimulus, and tail pinch, and pupillary constriction prior to administration and in week 13. Fore- and hind-limb grip strength was measured by a strain gauge device (Chatillon® Digital Force gauge). Responses to approach/touch, sharp auditory stimulus, and tail pinch were made while the animal was in a standard arena. Pupillary constriction was assessed after a beam of light was directed into each eye prior to removing the rats from the motor activity chambers because the darkened room in which the apparatus was located facilitated observing the response.

Motor activity evaluation
Following the abbreviated functional observational battery, assessment of motor activity (MA) was conducted prior to treatment and in week 13. Rats were individually tested in 1 of 30 identical, automated activity monitors (Coulbourn® Infrared Motor Activity System). Group and sex were counterbalanced across the monitors and time of day to the fullest extent possible. The infrared monitoring device enables measurement of 2 dependent variables: duration of movement and number of movements. A continuous movement was counted as 1 movement regardless of duration. Each test session was 60 minutes in duration, and the results were expressed for the total session, as well as for 6 successive 10-minute blocks.
Presence of diarrhoea and polyuria on the cage boards below the motor activity monitor were also recorded following each motor activity session.

Ophthalmoscopic examination
Ophthalmology examinations were conducted by a veterinary ophthalmologist. Both eyes were examined by focal illumination and indirect ophthalmoscopy. The examinations were conducted under subdued lighting after mydriasis had been produced with a 1 % tropicamide solution. On test day -15, the initial examination was performed on all rats received for the study, prior to selection and grouping. All surviving rats were examined on test day 85 prior to the scheduled sacrifice.

Haematology, clinical chemistry and urinalysis
A clinical pathology evaluation was conducted on all animals on test days 95 – 96. The day before collection of samples for the clinical pathology evaluation, the animals were placed in metabolism cages. These animals were fasted after 3 p.m. for at least 15 hours and urine was collected from each animal. Blood samples for haematology and clinical chemistry measurements were collected from the orbital sinus of each animal while the animal was under carbon dioxide anaesthesia. Blood samples for coagulation parameters were collected at sacrifice from the abdominal vena cava of each animal while the animal was under carbon dioxide anaesthesia.
Additional blood collected from the vena cava was placed in a serum tube, processed to serum, and frozen at approximately -80 °C. Serum will be discarded without analysis because further tests were not required to support experimental findings. Bone marrow smears were prepared at sacrifice from all surviving animals. Bone marrow smears were stained with Wright-Giemsa stain, but analysis was not necessary to support experimental findings. All blood samples were evaluated for quality by visual examination. Results were maintained in the study records and reported only if the sample was analysed.

The following haematological parameters were evaluated: Red blood cell count, red cell distribution width, haemoglobin, absolute reticulocyte count, haematocrit, platelet count, mean corpuscular (cell) volume, white blood cell count, mean corpuscular (cell) haemoglobin, differential white blood cell count, mean corpuscular (cell) haemoglobin concentration, microscopic blood smear examination, prothrombin time and activated partial thromboplastin time.

The following clinical chemistry parameters were evaluated: Aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, total bilirubin, urea nitrogen, creatinine, cholesterol, triglycerides, glucose, total protein, albumin, globulin, calcium, inorganic phosphorus, sodium, potassium and chloride.

The following urinalysis parameters were evaluated: Quality, colour, clarity, volume, osmolality, pH, glucose, ketone, bilirubin, blood, urobilinogen, protein and microscopic urine sediment examination.

Metabolite analysis
Additionally pooled urine samples for each treatment group were taken on Day 82 (males) and Day 83 (females) and individually plasma samples were taken on the same days to analyse N-MCX20 (N-acetyl glyphosate) and its possible metabolites, IN-B2856 (glyphosate) and IN-EY252 (N-acetyl AMPA).
The rat urine samples were prepared by adding 0.935 mL of 10 % formic acid to 0.05 mL of urine sample. The mixture was vortexed and 0.015 mL of $HClO_4$ was added. Finally, it was centrifuged after vortexing and the solution was transferred to a LC vial for LC/MS/MS analysis. The amount of 10 % formic acid was reduced to 0.185 mL when analyte concentrations in the samples were very low (i.e., samples from the 180 and 900 ppm groups) and a more sensitive LC/MS/MS method was used to analyse these samples.
The rat plasma samples were prepared by adding 0.05 mL of trifluoroacetic acid to 0.05 mL of plasma sample. The mixture was vortexed well and 0.4 mL of 10 % formic acid was added. Finally, it was centrifuged after vortexing and the solution was transferred to a LC vial for LC/MS/MS analysis.
The individual stock solutions of IN-MCX20, IN-B2856 and IN-EY252 were prepared in NANOpure® water. The stock solutions were then used to prepare calibration standards in the control rat urine or plasma matrices. Each calibration standard contained all three analytes at the similar concentrations.

Sacrifice and pathology
After approximately 90 days on study (test day 95 for males and 96 for females), all male and female rats from each exposure group (0, 180, 900, 4500 and 18000 ppm) were sacrificed and necropsied for evaluation of sub-chronic toxicity. Rats sacrificed by design were fasted after 3 p.m. on the afternoon preceding the day of sacrifice. The order of sacrifice for scheduled deaths was stratified across dose groups within each sex. Rats were euthanised by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all rats. Gross observations, final body weights and organ weights were recorded.

Organ weights
The following organs were weighed from all animals at the scheduled necropsy: Liver, kidneys, heart, spleen, thymus, adrenal glands, brain, testes, epididymides, ovaries (including oviducts), and uterus (including cervix). Group mean values and organ weight ratios (% body weight and % brain weight) were calculated.

Histopathology
The following organs were examined: Adrenals, aorta, bone with marrow (with femur and sternum), bone marrow smear, brain, epididymides, eyes, oesophagus, stomach, duodenum, jejunum, ileum, Peyer's Patches, caecum, colon, rectum, heart, kidneys, larynx, liver, lungs, lymph nodes (mandibular and mesenteric), nasal cavity, mammary gland (females only), ovaries, pancreas, peripheral nerve, pharynx, pituitary, prostate, salivary glands, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, testes, thymus, thyroid/parathyroids, trachea, urinary bladder, uterus, vagina and all gross lesions and masses.

Statistics
Significance was judged at $p<0.05$. Separate analyses were performed on the data collected for each sex.

Table 6.8.1.3.3.1-43: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Statistical analysis

Parameter	Preliminary Test	Method of Statistical Analysis	
		If preliminary test is not	If preliminary test is

		significant	**significant**
Body weight Body weight gain Food consumption Food efficiency Clinical pathology Organ weight	Levene's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Survival Incidence of FOB Descriptive parameters	None	Sequential application of Cochran-Armitage test for trend	
Motor activity Grip strength	Levene's test for homogeneity and Shapiro-Wilk test for normality	Repeated measures analysis of variance followed by Linear contrasts	Sequential application of the Jonckheere-Terpstra trend test

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSIS

The results of diet analysis from samples submitted mid-study demonstrate that the test substance was homogeneously distributed (coefficients of variance [CV] = 4 – 10 %) and was at the targeted concentrations (± 13.7 % of nominal) in all diets. Analysis of diet samples from the end of the diet also demonstrated that all diets were at targeted concentrations (± 17.8 % of nominal). No test substance was detected in any control diet samples.

The results of diet analysis from samples submitted at study start demonstrate that the test substance was stable in the diet under relevant storage conditions (up to 21 days at room temperature or refrigerated). A few of the stability samples were not within the targeted concentration range, including 0-day room temperature 180 ppm sample (65.0 % of nominal) and 14-day room temperature 18000 ppm sample (140.6 % of nominal). All other stability samples were within 20.6 % of nominal. Except for the 180 ppm diet, the mean concentrations in fresh diets collected at study start were within 15.8 % of nominal.

The low value (65 % of nominal) measured in the 0-day room-temperature 180 ppm diet sample did not represent instability of the test substance. This is supported by the fact that this low value was measured in the freshly collected sample stored frozen until analysed. In addition, other 180 ppm samples collected from the same diet mixing and analysed for stability assessment were within an acceptable range, and there was no reduction in concentration over time for samples stored at either room temperature or refrigerated for either concentration.

Since the stability analysis did not demonstrate any breakdown of test substance in the diet matrix, it can be assumed that the concentration measured in all samples was comparable to the concentration at the time of mixing. Therefore, the concentration and homogeneity of test substance in the samples of diets collected at study start, at the time of mixing, can be estimated based on the mean and CV of all of the stability samples. Based on these stability sample data, the mean concentration of test substance in the two diets was estimated to be at the targeted levels (± 9 %) for both diets.

Although the initial homogeneity, based on these stability sample data, was outside the Haskell targeted range (CV <10 %), this was driven mainly by one outlier value in each diet. Excluding the one high value for the 18000 ppm diet, the CV for this diet was within the Haskell targeted range. These data, combined with the homogeneity data from the mid-study samples, support that the diets were at the targeted range and fairly homogeneously mixed, especially at the higher concentrations. The homogeneity data in the 180 ppm diet may represent true lower homogeneity at this concentration. However, most of the samples at this concentration at study start fell within the acceptable targeted concentration range (± 20 % of nominal). Since this concentration was below the NOAEL, the analytical results are not critical in evaluating the results of exposure to the test substance.

Thus, the animals were considered to have received the targeted concentration of test substance in the diets over the course of the study.

B. MORTALITY

No test substance-related deaths occurred. One male in the 4500 ppm group was found dead on test day 42. This death was attributed to trauma and not to exposure to the test substance. All other rats survived to scheduled sacrifice.

C. CLINICAL OBSERVATIONS

No clinical observations were attributed to test substance exposure. All observed signs were those typically seen in rats of this age and in this type of study.

D. BODY WEIGHT

No adverse effects on body weight or body weight gain were observed in male rats. Final (test day 91) mean body weight in the 18000 ppm group was 92 % of control (not statistically significant) and mean body weight in this group was not significantly different from control on any test day. Statistically significantly lower overall (test day 0 – 91) body weight gain was observed in male rats in the 18000 ppm group (86 % of control). However, while mean weekly body weight gain was lower than control over most weekly intervals, the difference was only statistically significant over one weekly interval (test days 35 – 42), and body weight gain in this group exceeded that of control over two subsequent weekly intervals (not statistically significant). Therefore, this lower body weight gain was considered test substance-related but not adverse, as it didn't result in significantly lower mean body weight, and was not associated with a significant reduction in food consumption or food efficiency. No test substance-related effects on body weight or body weight gain were observed in any other male group. A statistically significant difference (reduction) in mean body weight gain was observed in the male 180 ppm group over test days 35 – 42. However, this difference was not considered test substance-related as there was no dose response.

No effects on body weight or body weight gain were observed in any female group. Final body weight and overall body weight gain in the female 18000 ppm group were 99 % and 97 % of control, respectively. Neither difference was statistically significant. No statistically significant difference in mean body weight or body weight gain was observed in any female dose group on any test day.

Lower body weight gain and/or body weight loss was observed in all male and female dose groups over the test day 77 – 84 interval. This lower weight gain is attributed to the stress of blood and urine collection on test days 82 (males) or 83 (females).

Table 6.8.1.3.3.1-44: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected body weight gain values

	Dose group [ppm]									
	Males					**Females**				
	0	**180**	**900**	**4500**	**18000**	**0**	**180**	**900**	**4500**	**18000**
Final body weight Day 91 [g]	583.2 ± 59.4	562.8 ± 60.1	574.5 ± 60.1	559.1 ± 35.4	533.9 ±50.2 (-8%)	286.8 ± 22.3	303.3 ± 21.7	294.3 ± 35.2	303.2 ± 28.8	282.8 ± 18.9
Mean body weight gain Day 35 – Day 42 [g]	32.9 ± 7.1	**↓17.7[$] ± 14.6**	↓25.4 ± 5.0	↓28.1 ± 5.4	**↓24.4[$] ± 4.0**	11.1 ± 10.0	↑11.8 ± 5.3	↑13.1 ± 9.7	↑12.4 ± 7.8	↓10.5 ± 6.4
Mean body weight gain Day 77 – Day 84 [g]	-0.9 ± 11.7	↑5.0 ± 6.2	↓-3.7 ± 7.0	↓-2.1 ± 4.1	↓-1.5 ± 4.9	-4.2 ± 4.6	↑0.4 ± 7.6	↑-0.6 ± 4.3	↑-4.0 ± 6.3	↑1.5 ± 4.9
Mean body weight gain Day 0 – Day 91 [g]	347.3 ± 51.9	↓327.5 ± 27.7	↓338.0 ± 46.1	↓324.1 ± 31.9	**↓298.6 [*] ± 36.8** (-14%)	110.9 ± 18.7	↑127.4 ± 17.0	↑120.5 ± 28.4	↑127.3 ± 21.9	↓107.3 ± 14.4

[*]: Statistically significant from control ($p \leq 0.05$; Dunnett/Tamhane-Dunnett test); [$]: Statistically significant from control ($p \leq 0.05$; Dunn's test)

A. FOOD COMSUMPTION; FOOD EFFICIENCY AND DAILY INTAKE

No adverse effects on food consumption were observed in male or female rats. Overall food consumption in the 18000 ppm male and female groups was 93 % and 105 % of control, respectively. Neither difference was statistically significant. Statistically significantly lower mean food consumption, compared to control, was observed over one weekly interval each in all male dose groups except the 900 ppm group. Statistically significantly higher mean food consumption was observed in the 4500 ppm and 18000 ppm female groups over one or three weekly intervals. None of these differences was considered test substance related as overall mean food consumption was not significantly different from control.

No adverse effects on food efficiency were observed in male or female rats. Overall food efficiency in the 18000 ppm male and female groups was 92 % of control in both males and females. Neither difference was statistically significant. Mean weekly food efficiency was significantly lower than control in 18000 ppm males over test days 7 – 14 and in 180 ppm males over test days 35 – 42. These differences were not considered adverse as overall

food efficiency was not significantly lower than control. No statistically significant differences in weekly food efficiency were observed in any female group during the study.
Mean daily intake of IN-MCX20 in males in the 0, 180, 900, 4500 and 18000 ppm groups was 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day, respectively. Mean daily intake of IN-MCX20 in females in the 0, 180, 900, 4500 and 18000 ppm groups was 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day, respectively.

Table 6.8.1.3.3.1-45: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected food consumption values

	Dose group [ppm]									
	Males					**Females**				
	0	**180**	**900**	**4500**	**18000**	**0**	**180**	**900**	**4500**	**18000**
Mean food consumption Day 28 – Day 35 [g]	30.6 ± 2.6	↓29.0 ± 1.6	↓29.5 ± 3.2	↓**27.9* ± 1.6**	↓**27.7* ± 2.7**	19.4 ± 2.1	↑21.2 ± 2.3	↑19.9 ± 2.4	↑**22.3$ ± 2.9**	↑20.4 ± 2.0
Mean food consumption Day 35 – Day 42 [g]	30.8 ± 3.5	↓**27.4* ± 2.9**	↓28.3 ± 3.0	↓28.2 ± 2.0	↓27.9 ± 2.6	18.9 ± 2.1	↑19.9 ± 1.6	↑20.5 ± 3.5	↑**22.7* ± 2.5**	↑21.2 ± 2.4
Mean food consumption Day 42 – Day 49 [g]	30.9 ± 2.9	↓28.1 ± 1.8	↓29.3 ± 3.4	↓28.9 ± 1.8	↓28.9 ± 2.7	19.3 ± 2.0	↑20.4 ± 1.7	↑19.8 ± 3.2	↑**21.8$ ± 2.0**	↑20.0 ± 1.7
Mean food consumption Day 0 – Day 91 [g]	29.8 ± 2.7	↓28.3 ± 1.7	↓28.3 ± 2.8	↓28.2 ± 1.6	↓27.8 ± 2.2	19.4 ± 1.7	↑20.2 ± 1.4	19.4 ± 2.1	↑21.2 ± 2.1	↑20.3 ± 1.3

*: Statistically significant from control (p≤0.05; Dunnett/Tamhane-Dunnett test); $: Statistically significant from control (p≤0.05; Dunn's test)

Table 6.8.1.3.3.1-46: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected food efficiency values

	Dose group [ppm]									
	Males					**Females**				
	0	**180**	**900**	**4500**	**18000**	**0**	**180**	**900**	**4500**	**18000**
Mean food efficiency Day 7 – Day 14 [g]	0.254 ± 0.031	↓0.245 ± 0.018	↑0.258 ± 0.034	↓0.244 ± 0.012	↓**0.226* ± 0.019**	0.130 ± 0.038	↓0.129 ± 0.042	0.130 ± 0.030	↑0.144 ± 0.036	↓0.111 ± 0.030
Mean food efficiency Day 35 – Day 42 [g]	0.152 ± 0.026	↓**0.086$ ± 0.084**	↓0.128 ± 0.021	↓0.142 ± 0.024	↓0.124 ± 0.012	0.080 ± 0.084	↑0.085 ± 0.036	↑0.085 ± 0.049	↓0.078 ± 0.047	↓0.071 ± 0.042
Mean food efficiency Day 0 – Day 91 [g]	0.128 ± 0.012	↓0.127 ± 0.005	↑0.131 ± 0.011	↓0.126 ± 0.007	↓0.118 ± 0.009	0.063 ± 0.011	0.069 ± 0.007	0.068 ± 0.009	0.066 ± 0.007	0.058 ± 0.006

*: Statistically significant from control (p≤0.05; Dunnett/Tamhane-Dunnett test); $: Statistically significant from control (p≤0.05; Dunn's test)

F. OPHTHALMOLOGICAL EXAMINATIONS

No ophthalmological observations were attributed to test substance exposure. All observed signs were those typically seen in rats of this age and in this type of study.

G. NEUROBEHAVIORAL EVALUATIONS

Abbreviated Functional Observational Battery

There were no test substance-related or statistically significant effects for fore- or hind-limb grip strength and for any behavioural parameter in either males or females administered dietary concentrations of 180, 900, 4500 or 18000 ppm of the test substance.

Motor Activity

There were no test substance-related or statistically significant effects on duration of movement or number of movements in either males or females administered dietary concentrations of 180, 900, 4500 or 18000 ppm of the test substance.

H. HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALIYIS

Haematology
There were no adverse changes in haematological parameters in male or female rats. The following statistically significant changes in mean haematological parameters were not adverse or not related to exposure to the test substance.
Mean monocyte count was mildly decreased at test day 95 in males fed 18000 ppm (mean was 61 % of the control group). Individual monocyte counts were within the overall range observed historically in similarly aged control rats, but several males fed 18000 ppm had values that were below the 95% historical reference interval. This change was of uncertain relationship to treatment. Regardless, decreases in monocyte counts have no clinical significance; therefore, this change was considered to be non-adverse.
Minimally increased mean neutrophil count at test day 95 in males fed 4500 ppm was considered to be unrelated to treatment and non-adverse, because it was not dose-related.

Table 6.8.1.3.3.1-47: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected haematological parameters

Parameter	Dose group [ppm]									
	Males					Females				
	0	**180**	**900**	**4500**	**18000**	**0**	**180**	**900**	**4500**	**18000**
Monocytes [×10³/µL]	0.28 ± 0.11	↓0.25 ± 0.06	↓0.27 ± 0.11	↓0.27 ± 0.11	↓**0.17* ± 0.07**	0.22 ± 0.10	↓0.20 ± 0.09	↓0.21 ± 0.06	0.22 ± 0.11	↑0.23 ± 0.13
Neutrophils [×10³/µL]	1.36 ± 0.41	↑1.72 ± 0.37	↑1.68 ± 0.40	↑**2.04* ± 0.88**	↑1.41 ± 0.51	1.42 ± 1.04	↓1.15 ± 0.58	↓1.10 ± 0.31	↓1.31 ± 0.54	↓1.15 ± 0.47

*: Statistically significant from control (p≤0.05; Dunnett/Tamhane-Dunnett test)

There were no treatment-related or statistically significant changes in coagulation parameters in male or female rats.

Clinical Chemistry
There were no adverse or treatment-related changes in clinical chemistry parameters in male or female rats. Decreased glucose at test day 95 in males fed 900 ppm was considered to be unrelated to treatment and non-adverse because it was not dose-related.

Table 6.8.1.3.3.1-48: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected clinical chemistry parameters

Parameter	Dose group [ppm]									
	Males					Females				
	0	**180**	**900**	**4500**	**18000**	**0**	**180**	**900**	**4500**	**18000**
Glucose [mg/dL]	116 ± 21	↓106 ± 16	↓**99$ ± 7**	↓115 ± 19	↓106 ± 20	96 ± 9	↑100 ± 10	↑97 ± 13	↑105 ± 16	↓92 ± 5

$: Statistically significant from control (p≤0.05; Dunn's test)

Urinalysis
There were no statistically significant or treatment-related changes in urinalysis parameters in male or female rats.

Urinalysis – Metabolic analysis
Rat Urine Sample Results
The urinary concentrations of IN-MCX20 increased with the increasing dietary levels of this test substance. Concentrations of IN-B2856 and IN-EY252 were detected above the limit of detection at higher dietary levels (900 to 18000 ppm) but at or below the limit of detection in urine samples from the 180 ppm dietary group. Further, the concentrations of these metabolites were much higher in urine samples from male rats than from corresponding female rats at 4500 and 18000 ppm. Neither IN-MCX20 nor either metabolite was detected in urine from control rats.

Rat Plasma Sample Results
The concentrations of IN-MCX20 also increased with the increasing dietary levels of this test substance. Concentrations of IN-MCX20 were <1.0 µg/mL for males and females in the 180 ppm dietary group. The concentrations increased from ~2 µg/mL up to ~14 µg/mL for the other dietary groups. In contrast to urine samples, little to none of IN-B2856 or IN-EY252 was detected for all dietary levels. Neither IN-MCX20 nor either metabolite was detected in plasma from control rats. The concentrations of IN-B2856 reported for 4500

ppm females were greater than those reported for the 18000 ppm females, although all concentrations were very low (close to the limit of detection). The reason for this could be that samples from different groups were analysed on different days, and the fact that the instrument responses were greater on the day the 4500 ppm rat plasma samples were analysed resulting in better quantitation, which is very common for LC/MS instruments. The standard deviation (± 0.26) for the 4500 ppm female samples indicates these results were not statistically significantly different from the 18000 ppm female samples.

Collectively, these analytical results of urine and plasma samples from rats exposed to INMCX20 confirm that this test substance is metabolised to small quantities of IN-B2856 and INEY252.

I. NECROPSY

There was no test substance-related mortality. A single male rat from the intermediate high-dose group (4500 ppm) was found dead on day 42. Important macroscopic findings were nose fracture and red discoloration of the stomach. The death was considered accidental and unrelated to test substance administration. All other rats survived until scheduled termination.

Organ weights

There were no treatment-related organ weight changes following the exposure period.

Gross pathology

At the terminal sacrifice, there were no treatment-related macroscopic findings.

Histopathology

Following the dosing phase, there were no treatment-related microscopic findings. Subacute/chronic inflammation, biliary hyperplasia and cholangio-fibrosis in the liver of a single intermediate high-dose male rat were considered spontaneous and incidental with no apparent relationship to the test substance exposure.

III. CONCLUSIONS

The no-observed-adverse-effect level (NOAEL) for male and female rats was 18000 ppm, equivalent to 1157 and 1461 mg/kg bw/day in males and females, respectively. The NOAEL is based on a lack of adverse, test substance-related effects in either sex at 18000 ppm, the highest concentration tested.

Assessment and conclusion by applicant:

Statistically significantly lower overall mean body weight gain (86 % of control) was observed in 18000 ppm males but was not considered adverse as it was not associated with a statistically significant difference in mean final body weight or in overall mean food consumption or food efficiency.

Despite the deviations in clinical chemistry (LDL, HDL, T3, T4 and TSH not determined), organ weights (prostate + seminal vesicles and coagulating glands), thyroid and pituitary gland not determined) and histopathology (cervix, coagulating glands, gall bladder and male mammary glands not examined) this study was conducted under GLP and was considered to be valid.

Therefore, the no-observed-adverse-effect level (NOAEL) for male and female rats was 18000 ppm, equivalent to 1157 and 1461 mg/kg bw/day in males and females, respectively. The NOAEL is based on a lack of adverse, test substance-related effects in either sex at 18000 ppm, the highest concentration tested.

Further, the study provides additional information about metabolism of N-acetyl glyphosate. It was shown that N-acetyl glyphosate was marginally metabolised to glyphosate and N-acetyl AMPA, as only very small quantities (close to LOD) were detected in urine and plasma samples at day 82/83.

Assessment and conclusion by RMS:

In contrast to the conclusion by the applicant, the RMS considers the statistically significant decrease in body weight gain (day 0-91) of 14% compared with controls in males dosed at 18000 ppm as treatment-related and adverse. This effect was associated with an 8% decrease in final body weight compared to controls. This dose level of 18000 ppm (1157 mg/kg bw/day) in males is considered the LOEAL and the NOAEL is 4500 ppm (283 mg/kg bw/day).

Additionally, N-acetyl glyphosate is metabolized to glyphosate and N-acetyl AMPA in small quantities.

B.6.8.1.3.4. Genotoxicity

Study 1

1. Information on the study

Data point	CA 5.8.1/041
Report author	[redacted]
Report year	2004
Report title	*Salmonella-Escherichia coli* / Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with N-Acetyl-Glyphosate
Report No	7353-101
Document No	VER04-COV-03
Guidelines followed in study	OECD 471 (1997)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 1) **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary

Executive summary
N-acetyl-glyphosate sodium salt, metabolite of glyphosate, was assessed for gene mutation in bacteria (Ames test). *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *Escherichia coli* strain WP2 uvrA were exposed to the test item, solvent (deionised water) and appropriate positive controls in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Dose levels were selected based on the results of a preliminary toxicity test in strains TA98 and WP2 uvrA, in which no cytotoxicity was observed up to 5000 µg/plate.
In the main mutagenicity study, two independent standard plate tests (plate incorporation method) were performed. Test item concentrations were in the range of 100 – 5000 µg/plate. After an incubation period of 52 ± 4 hours at 37 ± 2 °C, the bacterial background lawn was examined and the number of his^+ or trp^+ revertant colonies were counted.
There was no precipitation and no cytotoxicity observed for any tester strain at any concentration, neither in the presence nor in the absence of S9 mix.
In the first experiment, no increase in the mean number of revertant colonies was observed for any tester strain at any concentration, neither with nor without metabolic activation. In the confirmatory experiment, there was a 3.3-fold increase in the number of revertant colonies for strain TA 1537 in the absence of S9 mix. The increase was not dose-related and did not meet the criteria for a positive evaluation, therefore the observation was considered to be incidental. In addition, the mean value of spontaneous revertants obtained for the vehicle control with strain TA 98 in the absence of S9 mix was not within the acceptable range specified in the protocol. Therefore, a third experiment was performed for strain TA 98 in the absence of S9 mix. In the repeat mutagenicity test, all data were acceptable and there was no positive increase in the mean number of revertants per plate. In addition, the number of spontaneous revertants induced by the solvent control was as expected for all other strains in both experiments.
The number of revertant colonies induced by the positive controls were within the range of the laboratories historical control data, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.
Based on the experimental findings, N-acetyl-glyphosate sodium salt, a metabolite of glyphosate, did not induce gene mutations in bacteria and is therefore considered negative in the Ames standard plate test in the presence and absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: N-acetyl-glyphosate, sodium salt

Identification: Not specified

Description: White powder

Lot/Batch number: 123K5012

Purity: 84.3% (N-acetyl-glyphosate sodium salt) and 67.4% (N-acetyl-glyphosate as free acid)

Stability of test compound: The stability of the test item at storage conditions (at room temperature with desiccant) and the stability of the test item in solvent was not specified.

Solvent (vehicle) used: Deionised water

2. Control materials:

Negative control: A negative control was not employed in this study.

Solvent (vehicle) control: Deionised water

Solvent (vehicle)/final concentration: 0.05 mL per plate

Positive controls: Please refer to table below.

Strain	**Metabolic activation**	**Mutagen**	**Conc. [µg/plate]**
S. typhimurium strains			
TA 98	-S9	2-Nitrofluorene	1.0
	+S9	Benzo[a]pyrene	2.5
TA 100	-S9	Sodium azide	2.0
	+S9	2-Aminoanthracene	2.5
TA 1535	-S9	Sodium azide	2.0
	+S9	2-Aminoanthracene	2.5
TA 1537	-S9	ICR-191	2.0
	+S9	2-Aminoanthracene	2.5
E. coli strains			
WP2 uvrA	-S9	4-Nitroquinoline-N-oxide	1.0
	+S9	2-Aminoanthracene	25.0

3. Metabolic activation:

S9 mix was purchased from [redacted]. (Lot no. 1615 and 1626). The homogenate was prepared from the livers of male Sprague-Dawley rats that had been induced with a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bw. The S9 mix was thawed prior to each experiment and co-factors were immediately added to prepare S9 mix containing the following components:

S9 mix component	**Concentration**	**Unit**
Phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				**Bacteria batch checked for**	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(c) Preliminary cytotoxicity assay:

Plate incorporation test ± S9 mix:	
Concentrations:	6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, 5000 µg/plate*
Tester strains:	TA100 and WP2 uvrA
Replicates:	One plate was used for each condition.

*: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 67.4 %.

(d) Mutation assays:

Plate incorporation test ± S9 mix:	
Concentrations:	100, 333, 1000, 3000 and 5000 µg/plate*
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA
Replicates:	Triplicates

*: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 67.4 %.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 15 Mar – 04 May 2004
Finalisation date: 23 Dec 2004

2. Standard plate test (plate-incorporation test, SPT):

In tests with metabolic activation, an aliquot of 50 µL test solution, vehicle (deionized water) or positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix were added to 2 mL of molten top agar (supplemented with 0.5 mM histidine + 0.5 mM biotin or 0.5 mM tryptophan). In tests without metabolic action, 50 µL test solution, vehicle (deionized water) or positive control and 0.1 mL fresh bacterial culture were added to 2.5 mL of molten top agar. Afterwards, the mixture was vortexed and overlaid onto the surface of minimal bottom agar plates. After solidification, the plates were inverted and incubated for 52 ± 4 hours at 37 ± 2 °C. Each concentration and the controls were tested in triplicates. Following incubation, the bacterial background lawn was examined and the number of his^+ or trp^+ revertants colonies were counted.

3. Cytotoxicity

Toxicity was detected by a

- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

4. Statistics

For all replicate platings, the mean revertants per plate and the standard deviations were calculated. No further statistical analysis was performed.

5. Acceptance criteria

The assay was considered valid if the following criteria were met:

- The tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when treated with the solvent control. The acceptable ranges for the mean vehicle controls were 8 – 60 for TA 98, 60 – 240 for TA 100, 4 – 45 for TA 1535, 2 – 25 for TA 1537 and 5 – 40 for WP2 uvrA.
- The density of the tester strain cultures was $\geq 0.5 \times 10^9$ bacteria per mL and/ or had reached a target level of turbidity demonstrated to produce cultures with a density $\geq 0.5 \times 10^9$ bacteria per mL.
- The mean number of revertants induced by the positive controls were increased at least 3-fold over the mean value of the vehicle control for that strain.
- A minimum of three non-toxic concentrations was required to evaluate the assay data.

6. Evaluation criteria

A test item was considered positive (mutagenic) in the assay if the following criteria were met:

- There was an at least 2-fold increase in the mean number of revertant colonies per plate in at

least one tester strain when compared to the appropriate vehicle control for tester strains TA 98, TA 100 and WP2 uvrA.

- There was an at least 3-fold increase in the mean number of revertant colonies per plate in at least one tester strain when compared to the appropriate vehicle control for tester strains TA 1535 and TA 1537.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study.

B. CYTOTOXICITY

In the preliminary toxicity test, as well as in the main mutagenicity study, no cytotoxicity was observed with any tester strain up to the highest concentration of 5000 µg/plate, neither with nor without metabolic activation.

C. SOLUBILITY

Precipitation was not observed in any tester strain, at any concentration, neither with nor without S9 mix.

D. MUTATION ASSAY

In the first experiment, there was no increase in the number of his^+ or trp^+ revertant colonies observed for any strain at any concentration compared to the vehicle control, neither in the presence nor in the absence of S9 mix.
In the confirmatory experiment, there was a 3.3-fold increase in the number of revertant colonies for strain TA 1537 at 333 µg/plate in the absence of S9 mix. The increase was not dose-related and did not meet the criteria for a positive evaluation, therefore the observation was considered to be incidental. The RMS also notes that the number of revertant colonies were well within the range of the historical control data of the vehicle control.
In addition, the mean value of spontaneous revertants obtained for the vehicle control with strain TA 98 in the absence of S9 mix was not within the acceptable range specified in the protocol. Therefore, a third experiment was performed for strain TA 98 in the absence of S9 mix. In the repeat mutagenicity test, all data were acceptable and there was no positive increase in the mean number of revertants per plate. In addition, the number of spontaneous revertants induced by the solvent control was as expected for all other strains in both experiments.
The number of revertant colonies induced by the positive controls were within the range of the laboratories historical control data, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.

Table B.6.8.1.3.4.1-1: N-acetyl-glyphosate metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 2004), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	13	27	86	108	11	8	3	9	19	18
± *SD*	*± 3*	*± 5*	*± 13*	*± 10*	*± 7*	*± 3*	*± 2*	*± 2*	*± 2*	*± 2*
HCD[#] mean	15.9	26.4	85.4	91.9	15.8	12.9	7.7	9.5	16.4	17.2
± *SD*	*± 5.2*	*± 6.6*	*± 16.9*	*± 15.9*	*± 6.3*	*± 4.5*	*± 3.9*	*± 3.3*	*± 5.8*	*± 5.8*
[range]	*5 - 33*	*7 - 44*	*46 - 156*	*52 - 149*	*2 - 45*	*3 - 28*	*1 - 28*	*2 - 19*	*6 - 37*	*3 - 37*
Test item [µg/plate]										
100 mean	14	25	87	112	10	12	5	9	19	19
± *SD*	*± 3*	*± 10*	*± 6*	*± 6*	*± 4*	*± 2*	*± 2*	*± 1*	*± 4*	*± 2*
333 mean	10	22	94	108	10	12	4	11	18	19
± *SD*	*± 3*	*± 2*	*± 5*	*± 17*	*± 2*	*± 2*	*± 1*	*± 3*	*± 6*	*± 4*

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
1000 mean	12	26	88	115	8	9	6	9	17	17
± SD	*± 4*	*± 1*	*± 25*	*± 4*	*± 8*	*± 4*	*± 2*	*± 2*	*± 1*	*± 5*
3330 mean	15	30	94	107	10	15	7	7	17	18
± SD	*± 10*	*± 10*	*± 6*	*± 11*	*± 4*	*± 4*	*± 1*	*± 1*	*± 11*	*± 4*
5000 mean	16	24	98	114	9	14	4	5	15	17
± SD	*± 7*	*± 10*	*± 18*	*± 13*	*± 2*	*± 3*	*± 3*	*± 3*	*± 5*	*± 4*
Positive control										
[§] mean	290	249	1104	1335	985	249	581	121	161	406
± SD	*± 19*	*± 9*	*± 7*	*± 98*	*± 92*	*± 60*	*± 52*	*± 21*	*± 18*	*± 25*
HCD[#] mean	238.7	400.4	1054.9	706.4	749.8	143.4	835.6	118.6	242.5	595.0
± SD	*± 81.9*	*± 95.3*	*± 191.6*	*± 312.3*	*± 148.5*	*± 75.1*	*± 266.9*	*± 80.2*	*± 113.6*	*± 214.8*
[range]	*53 - 691*	*202 - 688*	*390 - 1515*	*111 - 2970*	*107 - 1291*	*68 - 691*	*97 - 1485*	*52 - 727*	*55 - 839*	*80 - 1098*

[§] = information on respective positive control is reported in Material and Method section I.A.2

[#] = Historical control data generated in the laboratory (time frame not specified) and based on at least 181 and 173 counts for the negative and positive control, respectively.

Table B.6.8.1.3.4.1-2: N-acetyl-glyphosate metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 2004), second experiment

Experiment 2: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	5	19	69	91	13	10	3	11	25	21
± SD	*± 2*	*± 2*	*± 2*	*± 9*	*± 1*	*± 0*	*± 2*	*± 5*	*± 5*	*± 5*
HCD[#] mean	15.9	26.4	85.4	91.9	15.8	12.9	7.7	9.5	16.4	17.2
± SD	*± 5.2*	*± 6.6*	*± 16.9*	*± 15.9*	*± 6.3*	*± 4.5*	*± 3.9*	*± 3.3*	*± 5.8*	*± 5.8*
[range]	*5 - 33*	*7 - 44*	*46 - 156*	*52 - 149*	*2 - 45*	*3 - 28*	*1 - 28*	*2 - 19*	*6 - 37*	*3 - 37*
Test item [µg/plate]										
100 mean	11	24	82	103	8	16	6	8	17	19
± SD	*± 2*	*± 3*	*± 7*	*± 0*	*± 5*	*± 5*	*± 2*	*± 2*	*± 3*	*± 4*
333 mean	11	23	76	114	12	16	10	11	18	24
± SD	*± 4*	*± 5*	*± 3*	*± 13*	*± 2*	*± 3*	*± 3*	*± 1*	*± 5*	*± 2*
1000 mean	14	19	85	102	10	8	8	10	18	18
± SD	*± 1*	*± 1*	*± 8*	*± 20*	*± 4*	*± 1*	*± 1*	*± 3*	*± 5*	*± 5*
3330 mean	14	25	78	99	12	16	4	10	19	24
± SD	*± 4*	*± 1*	*± 6*	*± 7*	*± 5*	*± 2*	*± 2*	*± 2*	*± 3*	*± 13*
5000 mean	12	19	76	109	16	16	6	11	19	24
± SD	*± 3*	*± 1*	*± 1*	*± 15*	*± 7*	*± 1*	*± 3*	*± 3*	*± 2*	*± 8*
Positive control										

Experiment 2: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
§ mean	343	113	1472	369	957	142	862	110	213	376
± *SD*	*± 60*	*± 16*	*± 108*	*± 41*	*± 24*	*± 16*	*± 78*	*± 15*	*± 31*	*± 25*
HCD# mean	238.7	400.4	1054.9	706.4	749.8	143.4	835.6	118.6	242.5	595.0
± *SD*	*± 81.9*	*± 95.3*	*± 191.6*	*± 312.3*	*± 148.5*	*± 75.1*	*± 266.9*	*± 80.2*	*± 113.6*	*± 214.8*
[range]	*53 - 691*	*202 - 688*	*390 - 1515*	*111 - 2970*	*107 - 1291*	*68 - 691*	*97 - 1485*	*52 - 727*	*55 - 839*	*80 - 1098*

§ = information on respective positive control is reported in Material and Method section I.A.2

= Historical control data generated in the laboratory (time frame not specified) and based on at least 181 and 173 counts for the negative and positive control, respectively.

Table B.6.8.1.3.4.1-3: N-acetyl-glyphosate metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 2004), third experiment

Experiment 3: Standard plate test (SPT)	
Strain	**TA 98**
Metabolic activation	**- S9**
Vehicle control	
Water mean	9
± *SD*	*± 5*
HCD# mean	15.9
± *SD*	*± 5.2*
[range]	*5 - 33*
Test item [µg/plate]	
100 mean	11
± *SD*	*± 2*
333 mean	11
± *SD*	*± 7*
1000 mean	14
± *SD*	*± 3*
3330 mean	12
± *SD*	*± 3*
5000 mean	13
± *SD*	*± 4*
Positive control	
§ mean	416
± *SD*	*± 17*
HCD# mean	238.7
± *SD*	*± 81.9*
[range]	*53 - 691*

§ = information on respective positive control is reported in Material and Method section I.A.2

= Historical control data generated in the laboratory (time frame not specified) and based on at least 181 and 173 counts for the negative and positive control, respectively.

III. CONCLUSIONS

Under the conditions of the present study, N-acetyl glyphosate sodium salt, metabolite of glyphosate, was negative for gene mutation in the Ames standard plate test in the presence and absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In the present study, N-acetyl glyphosate sodium salt was negative for gene mutation in bacteria (*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation. The study was conducted in compliance with GLP and according to OECD guideline 471 (1997), The study is therefore considered valid.

Assessment and conclusion by RMS:
It is agreed with the applicant that, in the present study, N-acetyl glyphosate was negative for gene mutation in bacteria (*S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) in the Ames pre-incubation test with and without metabolic activation.
The study was not available for the previous evaluation (RAR, 2015).

Study 2

1. Information on the study

Data point	CA 5.8.1/042
Report author	[redacted]
Report year	2004
Report title	Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells
Report No	7535-102
Document No	VER04-COV-02
Guidelines followed in study	OECD 473 (1997)
Deviations from current test guideline	Only 200 cells in metaphase were evaluated, whereas the currently valid OECD 473 (2016) recommends the evaluation of 300 metaphase cells per condition. In addition, cytotoxicity was evaluated based on mitotic indices, which is not recommended by the current guideline. Evaluation criteria specified in the study report were inconsistent with those specified in the guideline.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 1) **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the deviations noted above.

2. Full summary

Executive summary
N-acetyl-glyphosate, sodium salt, metabolite of glyphosate, was investigated in a chromosome aberration test in Chinese hamster ovary (CHO) cells. Duplicate cultures were exposed to the test item, negative (medium), solvent (water) and positive controls (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Test item concentrations in the range of 10 – 2800 µg/mL were screened for cytotoxicity by visual inspection, prior to selection of concentration levels used for chromosome analysis in the range of 960 – 2800 µg/mL.
The cells were treated for 3 hours in the presence and absence of metabolic activation and for 20 hours in the absence of metabolic activation at 37 ± 2 °C. In the 3-hour treatment schedule, the cells were washed following exposure and re-incubated afterwards. All cultures were harvested 20 hours after start of exposure and chromosome preparations were made. A total of 200 metaphases per condition (100 metaphases per culture) were scored for structural chromosome aberrations. In addition, the percentage of polyploid and endoreduplicate cells was scored among 100 metaphases. Cytotoxicity was assessed as mitotic index and evaluated for 1000 cells per culture.
Precipitation of the test item in solvent or medium was not observed for any concentration, neither in the

presence nor absence of S9 mix and independent of the treatment period. Cytotoxicity, evident as a slight reduction of the mitotic index, was observed after 3 hours of exposure at 1960 and 2800 µg/mL in the absence of S9 mix.
There was no statistically significant increase in the number of cells with chromosomal aberrations observed in any experiment at any concentration when compared to vehicle controls, neither in the presence nor in the absence of S9 mix. In addition, there was no increase in the frequency of cells in polyploidy and endoreduplication for any condition.
The frequency of aberrant metaphases in the negative, solvent and positive control cultures were within the range of the laboratory's historical control data, demonstrating the sensitivity of the test and the functionality of the S9 mix.
Based on the experimental results obtained in the present study, N-acetyl-glyphosate, sodium salt, metabolite of glyphosate, did not induce chromosomal aberrations in CHO cells *in vitro*, neither in the presence, nor in the absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: N-acetyl-glyphosate sodium salt

Identification:	Not specified
Description:	White powder
Lot/Batch #:	123K5012
Purity:	84.3 % (N-acetyl-glyphosate, sodium salt) and 67.4 % (N-acetyl-glyphosate as free acid)
Stability of test compound:	The stability of the test item under storage conditions (at room temperature with desiccant) was guaranteed until the expiry date 10 Feb 2006. The stability of the test item in solvent was at the responsibility of the sponsor.
Solvent (vehicle) used:	Water

2. Control Materials:

Negative control:	Culture medium
Solvent (vehicle) control:	Cell culture grade water (CCGW)
Positive control:	-S9 mix: Mitomycin C (MMC): 0.75 and 1.5 µg/mL for the 3-h treatment, 0.2 and 0.4 µg/mL for the 20-h treatment, respectively +S9 mix: Cyclophosphamide (CP): 7.5* and 12.5 µg/mL (*analysed for chromosomal aberrations)

3. Metabolic activation:

The metabolic activation system consisted of a rat liver post-mitochondrial fraction (S9 mix) and an energy producing system (NADP plus isocitric acid).
S9 mix was routinely prepared from the livers of rats, which received a single intraperitoneal injection of 500 mg/kg bw Aroclor 1254. Five days after treatment, the animals were sacrificed and the S9 homogenates were isolated. The metabolic activation system was prepared immediately before the experiment as follows:

S9 mix component	Concentration	Unit
Isocitric acid	10.5	mM
NADP, sodium salt	1.8	mM
S9	1.5	% (v/v)

4. Test organism:

Chinese hamster ovary (CHO) cells were used, established from an ovarian biopsy of a female Chinese hamster. The cell line was obtained from the laboratory of [redacted] and sub-cloned in the testing laboratory. Stock cultures were stored in liquid nitrogen. The cell line has an average cell cycle time of 12 – 14 hours and a modal chromosome number of 21. Stock cultures were maintained for up to 8 weeks after thawing. Twice during this period, the cells were screened for mycoplasma contamination.

5. Cell culture:

Medium:	McCoy's 5a culture medium supplemented with 10 % heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin G and 100 µg/mL streptomycin.
Incubation:	Cultures were incubated with loose caps in a humidified incubator at 37 ± 2 °C in an atmosphere of 5 ± 1.5 % CO2 in the air.
Cell culture establishment prior to exposure:	Cultures were initiated by seeding approx. 0.9 x 10^6 cells per 75 cm^2 flask 24 hours prior to treatment.

6. Test concentrations and number of replicates:

Metabolic activation	Duration of exposure (Fixation time)	Concentrations[§]	Replicates
-S9 mix	3 h (20 h)	10.0, 19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960*, 1370*, 1960* and 2800*[#] µg/mL	Duplicate
-S9 mix	20 h (20 h)	10.0, 19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960*, 1370*, 1960* and 2800*[#] µg/mL	Duplicate
+S9 mix	3 h (20 h)	10.0, 19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960*, 1370*, 1960* and 2800*[#] µg/mL	Duplicate

* Samples analysed for chromosomal aberrations; [#]: corresponding to > 10 mM of the free acid
[§]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 67.4 %.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 16 Mar – 22 Apr 2004
Finalisation date: 10 Sep 2004

2. Main cytogenicity test:

Treatment: Following cell culture establishment, duplicate cultures per condition were exposed to the test item, negative (culture medium), solvent (water) or positive control (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) in the presence and absence of metabolic activation.
The cells were treated for 3 hours with and without S9 mix and for 20 hours without S9 mix. After the 3-hour treatment period in the presence and absence of S9 mix, the cultures were washed with buffered saline, re-fed with culture medium and incubated for the remaining 17 hours until harvest. For the 20-hour treatment period in the absence of S9 mix, cells were harvested immediately following treatment.

Spindle inhibition: During the last 2 ± 0.5 hours of cultivation, cell division was arrested by addition of 0.1 µg/mL Colcemid to each culture.

Cell harvest: The cell cultures were trypsinised to collect mitotic and interphase cells. The cells were swollen in 75 mM potassium chloride and fixed in methanol : glacial acetic acid (3 : 1 v/v) fixative.

Slide preparation: Fixed cells were dropped on glass slides and air-dried. The slides were stained with 5 % Giemsa solution, then air-dried and mounted permanently.

Metaphase analysis: A total number of 200 metaphase cells per condition (100 metaphase cells per culture) were examined by microscopy. Cells were selected for good morphology and only cells with the number of centromers equal to the model number 21 ± 2 (range 19 – 23) were evaluated. At least 25 cells were analysed from those cultures that had greater than 25 % of cells with one or more aberrations. The cells were analysed for different type of chromosome and chromatide aberrations, including gaps, breaks and complex exchanges. The percentage of polyploid and endoreduplicate cells was scored among 100 metaphases.

Cytotoxicity: Prior to harvest of the cultures, visual observations of cytotoxicity were made, including

the assessment of confluency and the presence of mitotic (large, rounded cells) and dead, floating cells.
At scoring, the mitotic index of each culture was calculated based on the number of cells in metaphase observed per 1000 cells scored.

3. Statistics

The number and percentage of aberrant cells including and excluding gaps was analysed using a Cochran-Armitage test for linear trend and a Fisher's Exact test. The percentage of cells with aberrations in treated cells was compared to the results obtained for the vehicle control.
Statistical analysis was also performed for cells exhibiting polyploidy and/or endoreduplication in order to indicate significant events as indicators of possible induction of numerical aberrations; however, the test articles were evaluated only for structural aberrations and not for numerical aberrations in the protocol.

4. Acceptance criteria

The chromosome aberration test was considered acceptable if the following criteria were met:

- The vehicle control cultures contained less than approximately 5 % cells with aberrations.
- The positive control result was significantly higher than the vehicle control ($p \leq 0.01$).
- If the aberration results were negative and there was no significant reduction (≥ 50 %) in the mitotic index, the highest applicable dose (10 mM or 5 mg/mL) or a dose exceeding the solubility limit in culture medium must have been included.
- At least 3 dose levels for analysis of chromosome aberrations were included.

5. Evaluation criteria

A test substance was considered positive (clastogenic) in the chromosome aberration test if the following criteria were met:

- There was a significant increase in the number of cells with chromosomal aberrations observed at one or more concentrations.
- The linear trend evaluated the dose responsiveness. A dose-response was observed if a significant increase was seen at one or more concentrations.

A test substance was considered negative (not clastogenic) in the chromosome aberration test if no significant increase in the number of cells with chromosomal aberrations was observed at any concentration.

II. RESULTS AND DISCUSSION

A. ANALYTCAL DETERMINATIONS

Analytical determinations of the test substance in the solvent were not performed.

B. CYTOTOXICITY

Visual inspection of the cultures prior to harvest revealed no indication of cytotoxic effects. Cytotoxicity, evident as a slight reduction of the mitotic index, was observed after 3 hours of exposure for the two highest concentrations in the absence of S9 mix. The mitotic indices were reduced by 20 and 15 % at 1960 and 2800 µg/mL, respectively. There was no cytotoxicity at any concentration after exposure for 3 hours in the presence of S9 mix or after 20 hours of exposure in the absence of S9 mix.

C. SOLUBILITY

Precipitation of the test item in vehicle or culture medium was not observed for any condition, up to the highest tested concentration, neither in the presence, nor in the absence of metabolic activation.

D. CYTOGENICITY

There was no statistically significant increase in the number of cells with chromosomal aberrations observed in any experiment at any concentration when compared to vehicle controls, neither in the presence nor in the absence of S9 mix. In addition, there was no increase in the frequency of cells in polyploidy and endoreduplication for any condition.
The frequency of aberrant metaphases in the negative, solvent and positive control cultures were within the range of the laboratory's historical control data, demonstrating the sensitivity of the test and the functionality of the S9 mix.

Table B.6.8.1.3.4.2-1: Results of the cytogenicity test obtained with N-acetyl-glyphosate, sodium salt,

metabolite of glyphosate, after 3 hours of exposure in the presence and absence of S9 mix and after 20 hours of exposure in the absence of S9 mix ([redacted], 2004)

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Judge	Average % mitotic index	% Mitotic reduction
			no. structural aberrant cells[x]		% structural aberrant cells		% numerical aberrant cells				
			incl. gaps	excl. gaps	incl. gaps	excl. gaps	polyploid	endo-reduplicated			
Without metabolic activation; 3-hours treatment and 20-hours sampling											
Negative control											
Medium	0	200	0.0	**0.0**	0.0	**0.0**	**2.5**	**1.0**	negative	8.8	-
HCD[#] mean ± SD					1.4 ± 1.05	0.4 ± 0.56	1.3 ±1.44	0.0 ± 0.0			
range					0.5 - 3.5	0.0 - 1.5	0.0 - 4.0	0.0 ± 0.0			
Solvent											
CCGW	0	200	2.0	0.0	1.0	0.0	1.5	0.5	negative	11.00	0
HCD[#] mean ± SD					2.0 ± 1.39	0.7 ± 0.87	1.7 ± 1.33	0.3 ± 0.36			
range					0.0 - 4.0	0.0 - 2.5	0.0 - 3.0	0.0 - 1.0			
Test item [µg/mL]											
	960	200	7.0	1.0	3.5	0.5	2.5	0.5	negative	nd	nd
	1370	200	4.0	0.0	2.0	0.0	2.0	0.5	negative	nd	nd
	1960	200	4.0	0.0	2.0	0.0	3.0	0.5	negative	8.8	20
	2800	200	2.0	0.0	1.0	0.0	2.5	0.0	negative	9.4	15
Positive control [µg/mL]											
MMC	0.75	100	89	87	89	**87****	2.5	0.5	positive	nd	nd
HCD[#] mean ± SD					74.7 ± 10.20	72.2 ± 11.19	2.3 ± 1.91	0.0 ± 0.00			
range					55.0 - 88.0	52.0 - 88.0	0.0 - 5.0	0.0 - 0.0			
Without metabolic activation; 20-hours treatment and sampling											
Negative control											
Medium	0	200	2.0	1.0	1.0	0.5	2.5	0.0	negative	15.9	-
HCD[#] mean ± SD					2.5 ± 1.55	0.9 ± 1.17	2.7 ± 2.31	0.3 ± 0.90			
range					0.0 - 6.0	0.0 - 4.0	0.0 - 7.5	0.0 - 3.5			
Solvent											

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Judge	Average % mitotic index	% Mitotic reduction
			no. structural aberrant cells[x]		% structural aberrant cells		% numerical aberrant cells				
			incl. gaps	excl. gaps	incl. gaps	excl. gaps	polyploid	endo-reduplicated			
CCGW	0	200	7.0	0.0	3.5	0.0	3.0	0.0	negative	16.4	0
HCD[#] mean ± SD					3.7 ± 2.66	1.30 ± 1.50	1.8 ± 1.47	0.3 ± 0.92			
range					0.0 - 9.5	0.0 - 5.0	0.0 - 5.0	0.0 - 4.0			
Test item [µg/mL]											
	960	200	3.0	0.0	1.5	0.0	2.0	0.0	negative	nd	nd
	1370	200	1.0	1.0	0.5	0.5	2.5	0.0	negative	nd	nd
	1960	200	0.0	0.0	0.0	0.0	3.0	0.0	negative	nd	nd
	2800	200	2.0	0.0	1.0	0.0	3.0	0.0	negative	17.2	0
Positive control [µg/mL]											
MMC	0.20	100	92	89	92	**89****	3	0.5	positive	nd	nd
HCD[#] mean ± SD					61.2 ± 16.20	58.4 ± 16.22	2.0 ± 1.76	0.2 ± 0.36			
range					36.0 - 93.0	36.0 - 90.0	0.0 - 5.0	0.0 - 1.0			
With metabolic activation; 3-hours treatment, 20-hours sampling time											
Negative control											
Medium	0	200	5.0	0.0	2.5	0.0	4.0	4.5	negative	11.9	-
HCD[#] mean ± SD					2.5 ± 1.43	0.9 ± 0.88	2.2 ± 1.93	0.6 ± 0.74			
range					0.5 - 6.5	0.0 - 3.0	0.0 - 8.5	0.0 - 2.0			
Solvent control											
CCGW	0	200	2.0	0.0	1.0	0.0	3.0	1.5	negative	13.3	0
HCD[#] mean ± SD					2.2 ± 1.37	0.7 ± 0.85	1.9 ± 1.58	0.4 ± 0.51			
range					0.0 - 5.0	0.0 - 3.0	0.0 - 5.0	0.0 - 2.0			
Test item [µg/mL]											
	960	200	2.0	0.0	1.0	0.0	3.5	2.5	negative	nd	nd
	1370	200	3.0	1.0	1.5	0.5	3.0	2.0	negative	nd	nd

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity							Average % mitotic index	% Mitotic reduction
			no. structural aberrant cells[x]		% structural aberrant cells		% numerical aberrant cells		Judge		
			incl. gaps	excl. gaps	incl. gaps	excl. gaps	polyploid	endo-reduplicated			
	1960	200	7.0	2.0	3.5	1.0	3.5	2.5	negative	nd	nd
	2800	200	4.0	2.0	2.0	1.0	3.5	3.5	negative	14.1	0
Positive control [µg/mL]											
CP	7.50	100	62	53	62	**53****	3.5	2.5	positive	nd	nd
HCD[#] mean ± SD					57.5 ± 12.33	53.4 ± 12.93	3.3 ± 2.71	0.7 ± 1.05			
range					37.0 - 79.0	29.6 - 78.0	0.0 - 10.0	0.0 - 4.0			

HCD[#]: Historical control data from the laboratory's historical control range obtained in Jul - Dec 2003 based on 8 to 27 tests.

MMC: Mytomycin C, positive control without S9 mix; CP:cyclophosphamide, positive control with S9 mix

CCGW: Cell culture grade water, solvent control

[**]Statistically significantly increased (- gaps) when compared to the vehicle control, $p \leq 0.01$

nd: Not determined

[x]: Total number of chromosome aberrations from 200 metaphases scored

III. CONCLUSIONS

Under the conditions of the test, N-acetyl-glyphosate, sodium salt, metabolite of glyphosate, has no clastogenic potential in Chinese hamster ovary (CHO) cells, neither in the presence nor in the absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, N-acetyl-glyphosate, sodium salt was negative for induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without metabolic activation.

The test was performed under GLP conditions and in accordance with OECD guideline 473 (1997). There were only minor deviations when compared to OECD 473 (2016). The number of metaphases investigated was only 200, which was the number to be investigated recommended by the previous OCED 473 (1997). The study is therefore considered valid.

Assessment and conclusion by RMS:

It is agreed with the applicant that, in this test, N-acetyl glyphosate was negative for induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without metabolic activation.

The study is considered acceptable but with restrictions (reliable with restrictions) due to the deviation in number of scored metaphases. It is noted, however, that the study was conducted according to the applicable guideline (OECD 473, 1997) when conducting the study. The study was not available for the previous assessment (RAR, 2015).

Study 3

1. Information on the study

Data point	CA 5.8.1/043

Report author	[redacted]
Report year	2006
Report title	In Vitro Mammalian Cell Gene Mutation Test (CHO/HGPRT)
Report No	DuPont-20155
Document No	-
Guidelines followed in study	OECD 476 (1997), US EPA OPPTS 870.5300, EC Commission Directive 2000/32/EC Annex 4E-B17 (2000), JMAFF (1985)
Deviations from current test guideline	The newly introduced cytotoxicity parameter RS (relative survival) in OECD 476 (2016) and the adjusted cloning efficiency were not re-calculated, since no data on the number of cells after treatment were provided. In the current study, cytotoxicity was evaluated based on cloning efficiency after treatment (CE_1, survival) and after selection (CE_2, viability), in accordance with the previous guideline version. The RMS notes that the number of treated cells (assumed to be approximately 1 x 10^6 cells) and cells cultivated during expression and mutant selection phases ($\leq$ 10^6 cells and 2 x 10^5 cells, respectively) are neither in agreement with the OECD guideline applicable when the study was conducted (at least 10^6 cells at each stage in the test [OECD, 1997]) nor with the current OECD guideline (20 x 10^6 cells at treatment and at least 2 x 10^6 cells during expression and mutant selection [OECD, 2016]).
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid (Category 1) **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the deviation noted above.

2. Full summary

Executive summary

Disodium N-acetyl-N-(phosphonomethyl)glycine (IN-MCX20, batch: IN-MCX20-002, purity: 63 %) was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese hamster ovary (CHO K_1) cells. Duplicate cultures were exposed to the test item, solvent (water) and positive controls (ethylmethane sulfonate for cultures without S9 mix and benzo(a)pyrene for cultures with S9 mix) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Based on the results of a preliminary toxicity test, in which no substantial cytotoxicity was observed up to 2091 µg/mL (corresponding to 10 mM) with and without S9 mix, the concentrations for the main mutagenicity study were selected.

Test item concentrations in the range of 250 – 2091 µg/mL were used. After 5 hours of exposure with and without S9 mix, the cells were incubated for 7 days to allow expression of the mutant phenotype. The expression period was followed by a selection period, in which the cells were cultivated in medium supplemented with 6-thioguanidine for 9 days. Cytotoxicity was assessed as relative survival and relative viability after the expression and selection period, respectively.

There was no precipitation of the test item in solvent and in culture medium, neither in the presence nor absence of S9 mix. In addition, cytotoxicity, evident as a cloning efficiency $\leq$ 50 %) was not observed for any condition at any concentration. The relative cloning efficiency at the highest concentration of 2091 µg/mL was 115 % without S9 mix and 123 % with S9 mix. pH and osmolality were within an acceptable range.

Treatment with disodium N-acetyl-N-(phsphonomethyl)glycine did not induce a significant increase in the number of mutant colonies at any concentration, neither in the presence nor in the absence of metabolic activation. For all conditions and at all dose levels, mutant frequencies were below the minimum value for a positive response.

Mutant frequencies of the control cultures remained within the range of the laboratory's historical control data. The positive control mutagens ethylmethane sulfonate and benzo(a)pyrene induced mutant frequencies that were at least 3-fold above those of vehicle controls, demonstrating the functionality of the S9 mix and the sensitivity of the test.

Under the conditions of the test, there was no evidence for gene mutation in CHO-K_1 cells *in vitro*, neither in the presence nor in the absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Disodium N-acetyl-N-(phosphonomethyl)glycine

Identification:	IN-MCX20
Description:	White solid
Lot/Batch number:	IN-MCX20-002
Purity:	63 %
Stability of test compound:	The stability of the test item at storage conditions (under nitrogen in a desiccator) was guaranteed until the expiry date 25 Apr 2009. The stability of the test substance in vehicle was confirmed by analytical methods.

2. Control material:

Negative control:	The negative control was actually the solvent control.
Solvent (vehicle) control:	Water
Positive control:	- S9 mix: Ethylmethane sulfonate (EMS), 0.2 µL/mL in DMSO + S9 mix Benzo(a)pyrene (BaP), 4 µg/mL in DMSO

3. Metabolic activation:

S9 mix was purchased from [redacted]. The liver homogenate was produced from the livers of male Sprague-Dawley rats that were induced with Aroclor 1254. Immediately prior to use the S9 liver homogenate was thawed and mixed with co-factors as follows:

S9 mix component	**Concentration**	**Unit**
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organism:

Chinese hamster ovary (CHO-K_1) cells were obtained from American Type Culture Collection (ATCC) in Manassas, Virginia, USA. The cell stocks were stored in liquid nitrogen and routinely tested for mycoplasma contamination. Cells used in the mutation assay were within three to four subpassages from cleansing in pre-treatment medium supplemented with hypoxanthine, aminopterin and thymidine (HAT) in order to assure karyotypic stability.

5. Cell culture media:

Pre-treatment medium (HAT medium):	Ham's F12 medium supplemented with hypoxanthine, aminopterin and thymidine
Cultivation medium (F12FBS5-Hx):	Ham's F12 medium, supplemented with 5 % dialysed fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin
Treatment medium (± S9):	Cultivation medium (F12FBS5-Hx)
Selection medium:	Cultivation medium (F12FBS5-Hx) supplemented with 10 µM 6-thioguanidine (6TG)
Incubation:	At 37.5 ± 2 °C in a humidified atmosphere of 5 ± 2 % CO_2
Locus examined:	Hypoxanthin guanine phosphoribosyltransferase (HGPRT)

5. Test concentrations and number of replicates:

(a) Preliminary cytotoxicity and mutagenicity test:

Metabolic activation	**Duration of exposure**	**Concentrations[§]**	**Replicates**
± S9 mix	5 h	25, 50, 100, 250, 500, 750, 1000, 1500 and 2091[#] µg/mL	Triplicate plates from a single culture

[§]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.59.
[#]: corresponding to a concentration of 10 mM

(b) Main gene mutation test:

Metabolic activation	Duration of exposure	Concentrations[§]	Replicates
± S9 mix	5 h	250, 500, 1000, 1500 and 2091[#] µg/mL	Triplicate plates from duplicate cultures

[§]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.59.
[#]: corresponding to a concentration of 10 mM

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 22 Jun – 03 Aug 2006
Finalisation date: 13 Sep 2006

2. Preliminary cytotoxicity and mutagenicity test:
A preliminary test was performed to identify suitable dose levels for the main mutagenicity study. Cell cultures were established identically to the performance in the main mutagenicity test described below. The cells were exposed to nine test substance concentrations in the range of 25 - 2091 µg/mL for 5 hours in the presence and absence of S9 mix at 37 ± 2 °C. The highest concentration was equivalent to a 10 mM concentration. Following exposure, the cells were washed with Hank's Balanced Salt Solution (HBSS), re-incubated in fresh culture medium for approx. 20 hours, harvested and plated for determination of cell survival (cloning efficiency 1). After incubation for 7 days, the formed colonies were fixed, stained and counted.
Based on the results of the preliminary test, the test item concentrations for the main mutagenicity test were selected.

3. Main mutation assay:
Pre-treatment of cells:
For each test group, 5 x 10^5 cells were seeded into 25 cm^2 flasks and incubated for 16 - 24 hours prior to treatment. The RMS notes that the number of cells used at treatment was not determined. Assuming a doubling time of around 20 hours, approximately 1 x 10^6 cells would have been treated which is not in line with the OECD guideline ('see deviations').

Treatment:
On the day of treatment, the medium was exchanged. For treatment in the presence of S9 mix, 20 % of the medium were replaced by S9 mix. Duplicate cultures were exposed to the test item, vehicle (water) and positive controls (0.2 µL/mL ethylmethane sulfonate without S9 mix and 4 µg/mL benzo(a)pyrene with S9 mix) for 5 hours in the presence and absence of metabolic activation at 37 ± 2 °C. Test item concentrations ranged from 250 - 2091 µg/mL. Following exposure, the cells were washed with Hank's Buffered Salt Solution (HBSS) and the culturing was continued for another 18 – 24 hours. After the incubation period, the cells were trypsinised and counted. Triplicates of 100 cells/ 60 mm dish were seeded for the determination of survival (cloning efficiency 1) and duplicate cultures with ≤ 10^6 cells/ 100 mm dish were seeded for the expression of the mutant phenotype.

Expression period:
After treatment, duplicate cultures with ≤ 10^6 cells/ 100 mm dish were seeded and sub-cultured at 2-3 day intervals for a 7-day expression period. The RMS notes that the number of cells cultivated during the expression phase is not in agreement with the OECD guideline (see 'deviations'). After the expression period, each culture was divided. Triplicates of 100 cells/ 60 mm dish were seeded for the determination of viability (cloning efficiency 2) and five culture dishes with 2 x 10^5 cells/ 100 mm dish were seeded for the selection of mutants.

Selection period:
For the selection of mutants, five culture dishes with 2 x 10^5 cells/ 100 mm dish were seeded in medium enriched with 10 µM 6-thioguanidine (selection medium). The RMS notes that the number of cells cultivated during the mutant selection phase is not in agreement with the OECD guideline (see 'deviations'). After an incubation period of 9 days, the colonies were fixed, stained and counted.

4. Cytotoxicity:

Cloning efficiency CE_1 (survival)

The survival (cloning efficiency 1) of test item-treated cells relative to solvent controls was determined in parallel to the mutagenicity test. At the end of the exposure period, a sample of each cell culture was collected to assess survival of the cells. Triplicates of 100 cells/ 60 mm dish were seeded and incubated for 8 days. Afterwards, the colonies were fixed, stained and counted.

Cloning efficiency CE_2 (viability)

The viability (cloning efficiency 2) was determined in parallel to the selection of mutants. After the expression period, triplicates of 100 cells/ 60 mm dish were seeded in medium without 6-thioguanine to assess cell viability. After an incubation period of 9 days, the developed colonies were fixed, stained and counted.

5. Evaluation:

Cytotoxicity (cloning efficiency CE)

The number of colonies divided by the number of cells plated was calculated for each sample. The absolute cloning efficiency was determined for each test group, as well as the relative cloning efficiency in comparison to the solvent control group.

CE_1 (survival)

The cytotoxicity of the test substance after the exposure period was determined for each test group and is indicated as absolute and relative cloning efficiency (CE_1 and RCE_1, respectively).

CE_2 (viability)

The cytotoxicity of the test substance at the end of the expression period was determined for each test group and is given as absolute and relative cloning efficiency (CE_2 and RCE_2, respectively).

The cloning efficiency (CE, %) was calculated for each test group as follows:

$$CE_{absolute} = \frac{\text{Total number of colonies}}{\text{Total number of cells plated}} \times 100$$

$$RCE_x = \frac{CE_{absolute} \text{ of the test group}}{CE_{absolute} \text{ of the vehicle control group}} \times 100$$

Mutant frequency (MF)

The cloning efficiency of mutant colonies in selective medium divided by the cloning efficiency in non-selective medium measured for the same culture at the time of selection was calculated for each sample.

Uncorrected mutant frequency:

The uncorrected mutant frequency (MF_{uncorr}) was calculated for each test group as follows:

$$\text{MF uncorrected} = \frac{\text{Total number of mutant colonies}}{\text{Number of seeded cells}} \times 10^6$$

Corrected mutant frequency

The corrected mutation frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$\text{MF corrected} = \frac{\text{MF uncorrected}}{CE_2} \times 100$$

6. Statistics:

Statistical analysis was not performed.

7. Acceptance criteria:

The test was considered valid if the following criteria were met:

- The cloning efficiency of the vehicle control was > 50 % with a spontaneous mutant frequency within the range of the laboratories historical control data (0-25 mutants per 10^6 cloneable cells).
- The mutant frequency of the positive control was at least 3-fold as compared to the

concurrent vehicle control and exceeded 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data).
- At least 4 analysable concentrations showing mutants were required for evaluation.

6. 8. Evaluation criteria:

A test item was judged positive for gene mutation in mammalian cells if the following criteria were met:
- A mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at 2 or more consecutive concentrations of the test substance when compared to the solvent control.
- The observed increase in mutant frequency was accompanied by a concentration-related increase.
- A mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at the highest concentration only.

The test item was judged equivocal if a mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at any dose level other than the highest concentration.

The test item was judged negative for gene mutation in mammalian cells if a mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was not observed at any dose level.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS

Analytical determinations were performed for formulations 2.5, 10 and 20.91 mg/mL using a high performance liquid chromatography (HPLC) with MS/MS detection. The test item formulations were ± 15.6 % of the nominal concentrations, thus the test item was considered to be present at acceptable concentrations in the dosing solutions. In addition, the formulations were shown to be uniformly mixed (Coefficient of variation (CV) = 1, 2 and 4 % for 2.5, 10 and 20.91 mg/mL, respectively. There was no test substance in the 0 mg/mL sample. Stability of the test item in vehicle was confirmed for 5 hours at room temperature.

2. CYTOTOXICITY

In the preliminary cytotoxicity test, no cytotoxicity, evident as a cloning efficiency of ≤ 50 %, was observed. The relative cloning efficiency at the highest test item concentration (2091 µg/mL) was 78 % in the absence of S9 mix and 95 % in the presence of S9 mix. Osmolality and pH measurements of test substance in medium at 2091 µg/mL were found to be within an acceptable range. Based on these findings, the concentrations for the main mutagenicity study were selected to be in the range of 250 –2091 µg/mL, corresponding to 1.2 – 10 mM.

In the main mutagenicity experiment, there was as well no cytotoxicity observed at any dose level, neither in the presence nor absence of S9 mix. The relative cloning efficiency at the highest concentration of 2091 µg/mL was 115 % without S9 mix and 123 % with S9 mix. The pH in activated (+S9) and non-activated (-S9) cultures was 7.36 and 7.69 and comparable to the pH of solvent controls (7.45 and 8.04 in cultures with and without S9 mix, respectively). The observed changes in osmolality were ≤ 20 % and not considered significant.

3. SOLUBILITY

There was no precipitation observed up to the highest concentration tested, neither in the presence, nor absence of metabolic activation.

4. MUTANT FREQUENCY

Treatment with disodium N-acetyl-N-(phsphonomethyl)glycine did not induce a significant increase in the number of mutant colonies at any concentration, neither in the presence nor in the absence of metabolic activation. For all conditions and at all dose levels, mutant frequencies were below 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) and no dose-response relationship was evident.

Mutant frequencies of the solvent control cultures remained within the range of the laboratories historical control data. The positive control mutagens ethylmethane sulfonate and benzo(a)pyrene induced mutant frequencies that were at least 3-fold above those of vehicle controls, demonstrating the functionality of the S9 mix and the sensitivity of the test.

Table B.6.8.1.3.4.3-1: Results of the HGPRT gene mutation assay in mammalian cells with N-acetyl-glyphosate (IN-MCX20) ([redacted] 2006)

Test group	Total number of mutant colonies[$]	Mutant frequency (per 10^6 cells)	Cloning efficiency			
			CE_1 (survival, %)		CE_2 (viability, %)	
		corr.[#]	abs.	rel.	abs.	rel.[§]
Without metabolic activation; 5-hour exposure period						
Solvent						
Water	4	3.2	0.81	100	0.63	100
HCD mean ± SD		6.1 ± 6.6				
range		*0.0 - 24.0*				
Test item [µg/mL]						
250	6	5.5	0.82	101	0.54	86
500	10	7.6	0.83	102	0.66	105
1000	1	0.7	0.77	95	0.68	108
1500	7	4.8	0.74	91	0.73	116
2091	2	1.4	0.93	115	0.70	111
Positive control [µL/mL]						
EMS 0.2	470	352.5	0.57	70	0.67	106
HCD mean ± SD		216.0 ± 148.5				
range		*75.9 - 880.1*				
With metabolic activation; 5-hour exposure period						
Solvent						
Water	17	12.3	0.58	100	0.69	100
HCD mean ± SD		5.5 ± 5.4				
range		*0.0 - 19.0*				
Test item [µg/mL]						
250	16	10.4	0.56	96	0.77	112
500	2	1.4	0.50	86	0.73	106
1000	12	8.0	0.52	90	0.75	109
1500	17	12.6	0.66	113	0.67	97
2091	7	4.2	0.72	123	0.83	120
Positive control [µg/mL]						
(B(a)P) 4.0	135	103.8	0.17	29	0.65	94
HCD mean ± SD		206.3 ± 118.5				
range		*89.7 - 379.8*				

[#] = correction on the basis of the absolute cloning efficiency 2 at the end of the expression period

[$]: number of mutant colonies 9 days after seeding 2 x 10^5 cells/100 mm dish, total of 5 cultures

[§]: calculated with total cloning efficiency data (solvent control = 100 %)

HCD: Historical control data, generated in the laboratory from 1989 - 2005

III. CONCLUSIONS

Based on the experimental findings and under the conditions of the test, Disodium N-acetyl-N-(phosphonomethyl)glycine did not induce gene mutations in the HGPRT locus of CHO-K_1 cells, neither in the presence nor in the absence of metabolic activation and is therefore considered negative for mutagenicity in mammalian cells *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In the present study, Disodium N-acetyl-N-(phosphonomethyl)glycine was negative for mutagenicity at the HGPRT locus in CHO-K_1 cells with and without metabolic activation.
The study was conducted in compliance with GLP and according to OECD guideline 476 (1997). There were only minor deviations when compared to OECD 476 (2016), which were considered to not compromise the validity of the study. The study is therefore considered valid.

Assessment and conclusion by RMS:
It is agreed with the applicant that, in this study, disodium N-acetyl-N-(phosphonomethyl)glycine was negative for mutagenicity at the HGPRT locus in CHO-K_1 cells with and without metabolic activation. However, due to the deviations noted at the beginning of the study summary, the study is considered to be acceptable but with restrictions (reliable with restrictions) only.
The study was not available for the previous evaluation (RAR, 2015).

Study 4

1. Information on the study

Data point	CA 5.8.1/044
Report author	[redacted]
Report year	2006
Report title	IN-MCX20: Mouse Bone Marrow Micronucleus Test
Report No	[redacted]-20154
Document No	-
Guidelines followed in study	OECD 474 (1997), US EPA OPPTS 870.5395 (1998), EEC Directive 2000/32/EC B12 (2000), JMAFF 12 Nousan (2000)
Deviations from current test guideline	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. However, in the present study only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity observed. In addition, evaluation and acceptance criteria specified in the study report were different from those recommended in the guideline.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Conclusion GRG: Valid (Category 1) Conclusion AGG: The study is considered acceptable but with restrictions (reliable with restrictions).

2. Full summary

Executive summary
Disodium N-acetyl-N-(phosphonomethyl)glycine was investigated in a micronucleus test in male and female Crl:CD1 (ICR) mice. Based on the results of a preliminary toxicity study, in which no toxicity was observed up to a limit dose of 2000 mg/kg bw, dose levels for micronucleus tests were selected. Groups of 5 mice per sex per dose level were administered a single dose of 500, 1000 or 2000 mg/kg bw by oral gavage. For the high dose group, 2 additional mice/sex/sampling time point were treated and served as backup (scoring of micronuclei was not performed). Similar constituted groups received the vehicle (Nanopure® water) or the positive control (30 mg/kg bw cyclophosphamide).
Body weights of the animals were collected before dosing, 24 hours after treatment and prior to scheduled necropsy. The animals were observed for clinical signs of toxicity and mortality 1, 3-5 and 24 hours after treatment and prior to scheduled necropsy. About 24 and 48 hours after dosing, the animals of the test item-treated and control groups were sacrificed and bone marrow smears were prepared. For animals of the positive control group, bone marrow was sampled 24 hours after dosing. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. In addition, the ratio of PCE /

normochromatic erythrocytes (NCE) was determined for 1000 erythrocytes.
Treatment with disodium N-acetyl-N-(phosphonomethyl)glycine induced no mortality in the animals and no clinical signs of toxicity were noted at any dose level. In addition, body weight and body weight gain were not affected in any dose group at any sampling time point. Based on the ratio of PCE / NCE, there was no evidence for bone marrow toxicity.
There was no statistically significant and no biologically relevant increase in the frequency of mPCE when compared to vehicle control animals at any dose level and for any sampling time.
Incidences of mPCE in solvent and positive control animals were as expected and fell within the range of the laboratory's historical control data, demonstrating the validity and sensitivity of the test system.
Based on the experimental findings of the present study and under the conditions of the test, disodium N-acetyl-N-(phosphonomethyl)glycine did not induce micronuclei in the bone marrow of male and female mice *in vivo*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Disodium N-acetyl-N-(phosphonomethyl)glycine

Identification:	IN-MCX20
Description:	White solid
Lot/Batch #:	IN-MCX20-002
Purity:	63 %
Stability of test compound:	The stability of the test item at storage conditions (under nitrogen in desiccator) was guaranteed until the expiry date 25 Apr 2009. The stability of the test substance in vehicle was confirmed by analytical methods.
Solvent (vehicle) used:	Nanopure® water

2. Control materials

Solvent (vehicle) control:	Nanopure® water
Positive control:	Cyclophosphamide, 30 mg/kg bw in Nanopure® water

3. Test animals:

Species:	Mouse
Strain:	Crl:CD1 (ICR)
Sex:	Male and female
Source:	[redacted]
Age at dosing:	Approx. 7 weeks
Mean weight at dosing:	26.3 – 34.2 (males) and 20.6 – 27.1 (females)
Acclimation period:	At least 5 days
Diet/Food:	LLC Certified Rodent LabDiet® 5002 (PMI® Nutrition International), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually

4. Environmental conditions:

Temperature:	18 - 26 °C
Humidity:	30 - 70 %
Air changes:	Not specified
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	1500 and 2000 mg/kg bw
Concentrations:	Not specified
Dose volume:	Not specified
Number of animals:	3 males/group
Route of administration:	Oral gavage

b) Main micronucleus test

Dose levels:	500, 1000 and 2000 mg/kg bw (Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor.)
Concentrations:	50, 100 and 200 mg/mL
Dose volume:	10 mL/kg bw
Number of animals:	5/ sex/group and additional 2/sex/sampling time point as backup for the high dose group (additional animals were not used for scoring of micronuclei)
Route of administration:	Oral gavage

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 24 May – 28 Jun 2006
Finalisation date: 28 Aug 2006

2. Animal assignment and treatment:

Preliminary toxicity study:

In a preliminary range-finding test, groups of 3 male mice were administered a single dose of 1500 or 2000 mg/kg bw by oral gavage. The animals were observed for clinical signs of toxicity and mortality immediately after dosing and daily for two days. Based on the results of the preliminary toxicity study, the dose levels for the micronucleus test were selected.

Main micronucleus test:

Groups of 5 mice per sex and dose level were administered a single dose of 500, 1000 or 2000 mg/kg bw. For the high dose group, 2 additional mice/sex/sampling time point were treated and served as backup (scoring of micronuclei was not performed). The test substance was dissolved in Nanopure® water and administered by oral gavage at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of 5 mice/sex received the vehicle (Nanopure® water) or the positive control (30 mg/kg bw cyclophosphamide).
Individual body weights were collected before dosing and 24 and 48 hours after treatment. The animals were observed for clinical signs of toxicity and mortality 1, 3-5 and 24 hours after treatment and prior to scheduled necropsy. About 24 and 48 hours after dosing, the animals of the test item-treated and vehicle control groups were sacrificed by CO_2 asphyxiation and bone marrow smears were prepared. Animals of the positive control group were sacrificed 24 hours after administration.

3. Slide preparation:

After sacrifice, both femurs of the animals were removed, and marrow was aspirated with the aid of a syringe containing fetal bovine serum (FBS). After centrifugation, the pellet was re-suspended in FBS and a small drop was placed on pre-cleaned microscope slides. Smears were made using a Mini Prep® blood smearing instrument. At least 3 slides were prepared for each animal. The slides were air-dried, fixed in methanol and stained in acridine orange.

4. Slide evaluation:

Slides were coded and evaluated by fluorescent microscopy. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. Cells containing more than one micronucleus were scored as single micronucleated PCE (mPCE). In addition, the proportion of PCE among 1000 erythrocytes, expressed as PCE / normochromatic erythrocytes (NCE) ratio, was determined.
If no increase in the incidence of mPCE was observed at the 24-hour sampling time point at any dose level,

slide evaluation for the 48-hour sampling was performed for animals of the high dose and control group only.

5. Statistics:

Data for the amount of micronucleated polychromatic erythrocytes (mPCE) among 2000 erythrocytes and the ratio of PCE among normochromatic erythrocytes (NCE) were transformed prior to analysis using an arcsine square root or Freeman-Tukey function. Transformed data for PCE and MNPCE frequencies were analyzed separately for normality of distribution and equal variance using the Shapiro-Wilk and Levene's tests, respectively.

For those data that were normally distributed and had equal variance, parametric statistics (e.g. analysis of variace (ANOVA) and Dunnett's test were performed using the transformed data. For those data that were normally distributed but had unequal variance, a robust ANOVA and unequal-variance Dunnett test was done. For those data that were not normally distributed, nonparametric statistics (e.g., Kruskal-Wallis and Dunn's tests) utilizing non-transformed data was performed. The individual animal was considered the experimental unit. All data analyses was one-tailed and conducted at a significance level of 5 %.

6. Acceptance criteria:

The study was considered valid if the following criteria were met:

- In the vehicle control group, the mean frequency of micronucleated polychromatic erythrocytes (mPCE) was within the range of the laboratory's historical control range.
- The positive control induced a statistically significant increase in the frequency of mPCE as compared to the vehicle control group.

7. Evaluation criteria:

The test item was considered positive if the following criteria were met:

- The group mean number of micronucleated polychromatic erythrocytes (mPCE) was statistically significantly increased at one or more concentrations when compared to the concurrent vehicle control value.
- An accompanying statistically significant dose-response increase in mPCE was observed.

The test item was considered negative if the following criteria were met:

- There was no statistically significant, dose-related increase in the group mean mPCE above the concurrent vehicle control at any concentration.
- The mPCE values were within reasonable limits of the recent (past 3 years) laboratory historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The dosing formulations were analysed by high performance liquid chromatography (HPLC) and UV/Vis detection. The data indicated that the achieved concentration of test substance in vehicle was ± 14 % of the nominal concentration, which was considered acceptable. In addition, the formulations were shown to be uniformely mixed (Coefficient of variation (CV) = 6, 9 and 3 %) for 50, 100 and 200 mg/mL, respectively. There was no test substance in the 0 mg/mL sample. Stability of the test item in vehicle was confirmed for 5 hours at room temperature.

B. PRELIMINARY TOXICITY STUDY

In the range-finding experiment, no clinical signs of toxicity or mortality were observed.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

There were no clinical signs of toxicity observed at any time point or at any dose level in male or female animals exposed to the test substance.

Body weight and body weight gain:

There were no significant changes in body weight or body weight gain in either male or female animals of any test group.

Evaluation of bone marrow slides:
The ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) was not affected upon treatment with the test substance at any dose level and sampling time point, indicating that no bone marrow toxicity occurred.
In addition, there was no statistically significant and no biologically relevant increase in the frequency of mPCE when compared to vehicle control animals at any dose level and for any sampling time.
Incidences of mPCE in solvent and positive control animals were as expected and fell within the range of the laboratory's historical control data, demonstrating the validity and sensitivity of the test system.

Table B.6.8.1.3.4.4-1: Summary of genotoxicity data obtained with disodium N-acetyl-N-(phosphonomethyl)glycine (IN-MCX20) in the micronucleus test in mice ([redacted], 2006)

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD / 2000 PCE	PCE / NCE	mPCE ± SD / 2000 PCE	PCE / NCE
Solvent control						
Deionised water	/	24 h	1.2 ± 1.3	1.003 ± 0.460	0.2 ± 0.4	1.042 ± 0.457
	/	48 h	0.4 ± 0.6	1.868 ± 0.820	1.0 ± 1.7	1.420 ± 0.370
HCD mean ± SD	/	Not specified	3 ± 2	1.20 ± 0.43	3 ± 2	1.32 ± 0.49
range	/		*0 - 8*	*0.198 - 2.425*	*0 - 10*	*0.144 - 2.731*
Test item [µg/mL]						
Test item	500	24 h	1.0 ± 0.7	0.833 ± 0.608	1.4 ± 1.1	1.320 ± 0.310
	1000	24 h	1.0 ± 1.2	1.360 ± 0.170	0.2 ± 0.4	0.972 ± 0.350
	2000	24 h	1.0 ± 1.0	1.220 ± 0.485	2.0 ± 2.3	1.635 ± 0.444
	2000	48 h	1.4 ± 1.5	1.433 ± 0.590	1.8 ± 1.3	1.610 ± 0.484
Positive control [µg/mL]						
CPA	30	24 h	**17.2 ± 4.3***	1.213 ± 0.239	**17.4 ± 6.9***	1.187 ± 0.255
HCD mean ± SD	not specified	24 h	27 ± 15	1.29 ± 0.42	22 ± 11	1.38 ± 0.48
range	not specified		*8 - 81*	*0.364 - 2.623*	*4 - 67*	*0.143 -2.534*

PCE: polychromatic erythrocytes; mPCE: micronucleated polychromatic erythrocytes; NCE: normochromatic erythrocytes
HCD: Historical control data, generated in the laboratory in 16 male and 16 female studies, conducted from 2001 - 2005
CPA: cyclophosphamide; * statistically significant when compared to solvent control

III. CONCLUSIONS
Based on the results of the present study and under the conditions of the test, disodium N-acetyl-N-(phosphonomethyl)glycine did not induce micronuclei in bone marrow of male and female mice and is therefore considered negative for clastogenicity *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In the present study, disodium N-acetyl-N-(phosphonomethyl)glycine was negative for clastogenic and aneugenic effects in the bone marrow of male and female Crl:CD1 (ICR) mice *in vivo*.
The study was conducted under GLP conditions and in accordance with OECD guideline 474 (1997). Only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). Bone marrow toxicity, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, or systemic toxicity were not observed. However, the test was performed at limit dose levels in line with the current guideline. The deviations were considered to be of minor degree and to not compromise the validity of the study. The study is therefore considered valid.

Assessment and conclusion by RMS:
It is agreed with the applicant that, in this study, N-acetyl-N-(phosphonomethyl)glycine was negative for clastogenic and aneugenic effects in the bone marrow of male and female Crl:CD1 (ICR) mice *in vivo*. The study is considered to be acceptable but with restrictions (reliable with restrictions) due to the deviation in the number of scored polychromatic erythrocytes. It should however be noted that bone marrow exposure was not shown that as there was no effect on the PCE/NCE ratio and no systemic exposure was observed.
The study was not available for the previous evaluation (RAR, 2015).

B.6.8.2. Supplementary studies on the active substance

B.6.8.2.1. Immunotoxicity

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	2012
Report title	Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice
Report No	[redacted]-50393
Document No	M-651162-01-1
Guidelines followed in study	US EPA OPPTS 870.7800 (1998)
Deviations from current test guideline (No equivalent EU or OECD Test Guideline. US EPA specific guideline developed under FIFRA)	The study was performed according to EPA OPPTS 870.7800 to meet and evaluate US EPA specific endpoint for immunotoxicity under the Federal Insecticide, Fungicide, and Rodenticide Act. No equivalent OECD study guideline exists.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes, conducted under GLP
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: Acceptable.

2. Full summary

The potential immunotoxicity of glyphosate was evaluated after repeated dietary administration to B6C3F1 mice. Four groups of 10 female mice were offered diets containing glyphosate concentrations of 0, 500, 1500 or 5000 ppm (equivalent to 0, 150, 449, and 1448 mg/kg bw/day) and for 28 consecutive days. A further group of 10 females were used as positive immunosuppressive control group. These mice received basal diet for 28 days and were treated with an intraperitoneal (i.p.) injection of 50 mg/kg bw/day cyclophosphamide monohydrate (CPS) once daily for four consecutive days (study days 24 - 27).
The animals were checked twice daily for mortality and moribundity, and once daily for clinical signs. Detailed clinical examinations were performed once per week. Body weights were recorded twice weekly. Food consumption was recorded in weekly intervals, and food intake was calculated for the corresponding body weight intervals. Blood samples for IgM antibody analysis were collected from all mice at scheduled necropsy. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Spleens and thymus were weighed and specified tissues preserved.
There were no test substance-related effects on survival, clinical observations, body weight, food consumption, as well as any gross pathological changes. There were no test substance-related effects on spleen or thymus weights (absolute or relative to final body weight), spleen cellularity, or the T-cell dependent antibody response (TDAR), as measured by the splenic antibody-forming cell (AFC) IgM Specific Activity (AFC/10^6 spleen cells) and Total Spleen Activity (AFC/spleen), at any dosage level tested.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:	
Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	GLP-0807-19475-T
Purity:	95.11 % (dried)
Stability of test compound:	Expiry date: 2011-06-10
Vehicle and/ or positive control:	Basal diet Cyclophosphamide monohydrate
Test animals:	
Species:	Mouse
Strain:	B6C3F1/Crl
Source:	[redacted]
Age:	Approx. 37 days (on arrival)
Sex:	Female
Weight at dosing:	16.5 – 20.0 g
Acclimation period:	14 days
Diet/Food:	Certified Rodent LabDiet® # 5002 (meal) (PMI Nutrition International, LCC.), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel, wire-mesh cages suspended above cage-board.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 50 ± 20 % Air changes: 10/hour Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2010-10-05 to 2010-11-16

Animal assignment and treatment
In a 28-day oral immunotoxicity study groups of 10 female B6C3F1/Crl mice received daily dietary doses of 0, 500, 1500 and 5000 ppm glyphosate (equivalent to 0, 150, 449 and 1448 mg/kg bw/day).
A further group of 10 females were used as positive immunosuppressive control group. These mice received basal diet for 28 days and were treated with an intraperitoneal (IP) injection of 50 mg/kg bw/day CPS once daily for four consecutive days (study days 24 - 27).

Test diets were prepared weekly and stored at room temperature. For the vehicle and positive control groups an appropriate amount of basal diet was weighed into a plastic storage bag. For the test substance groups 500 g of basal diet was weighed (pre-mixture). An appropriate amount of glyphosate was weighted into a mortar, mixed with a small amount of the pre-mixture basal diet, and ground until uniform. This admixture was transferred to a Hobart mixer and mixed with the remainder of the pre-mixture basal diet for five minutes. The resultant mixture was then transferred to a V-blender with a sufficient amount of basal diet to achieve the correct diet concentration and mixed for an additional 10 minutes using an intensifier bar during the first and last three minutes of mixing to ensure a homogeneous mixture. The test diets were prepared from the lowest to highest concentration. The stability and homogeneity of the test substance in the diet was determined in an in-house

stability study at 450 and 5500 ppm. Analyses for achieved concentrations on the test diets were done during study weeks 0 and 3.

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

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed once a week during the study period, beginning one week prior to randomisation, and on the day of scheduled necropsy.

Body weight
Individual body weights were recorded twice weekly, beginning approximately one week prior to randomization, at the time of animal selection for randomization, on study day 0, and just prior to the scheduled necropsy. Mean body weights and mean body weight changes were calculated for the corresponding intervals.

Food consumption and test substance intake
The quantity of food consumed was recorded for each animal weekly, beginning approximately one week prior to randomization, and just prior to the scheduled necropsy. Food intake was calculated as g/animal/day for the corresponding body weight intervals. The mean amounts of glyphosate consumed (mg/kg bw/day) per dose group were calculated from the mean food consumed (g/kg of body weight/day) and the appropriate target concentration of glyphosate in the food (mg/kg of diet).

Serum collection for possible IgM antibody analysis
All animals were immunised with an intravenous injection of sRBC on study day 24. For determination of the possible extent of the suppression of IgM antibody production blood samples were collected from all animals at scheduled necropsy and processed to serum. Following euthanasia by carbon dioxide inhalation, approximately 0.75 mL of blood was collected from the inferior vena cava of each mouse into a tube containing no anticoagulant and allowed to clot. Serum was obtained and aliquots of approximately 150 µL (including any remainder serum) were transferred to cryovials and stored frozen (approximately -70 °C). The analysis was not conducted since there was no immunosuppressive effect evident in the spleen count, specific activity and total spleen activity.

Sacrifice and pathology
A complete necropsy was conducted on all animals at scheduled termination or on animals that died or were sacrificed during the study period. Any macroscopic findings were recorded. The following organ weights were determined from all animals surviving to scheduled termination: spleen and thymus. The ratio of organ weights to final body weights were calculated.
Tissue samples were taken from the spleen and thymus. Spleen samples were placed in EBSS/HEPES buffer. Thymus samples were preserved in 10 % neutral-buffered formalin.

Spleen processing for immunotoxicological evaluation

Spleens were collected from all animals at the scheduled necropsy (study day 28) immediately following blood collection. Individual spleens were placed into individual tared tubes containing EBSS with 15 mM HEPES, supplemented with gentamicin as a bacteriostat, and maintained on ice. Each tube was then weighed to provide a "wet" weight for each spleen. Spleen samples from Groups 1 - 4 animals were randomized and coded for antibody-forming cell (AFC) analysis. Spleen samples from Group 5 were labelled as positive control samples for analysis. The spleen samples were placed on crushed ice until procession for AFC analysis.
The spleen samples were processed into single-cell suspensions. The cell suspensions were then centrifuged and resuspended in EBSS with HEPES. Spleen cell counts were performed using a Model Z1™ Coulter Counter®. Viability of splenocytes was determined using propidium iodide and the Coulter® EPICS® XL-MCL™ Flow Cytometer. The AFC assay served to determine the number of specific IgM antibody-forming cells directed towards sRBC and was a modification of the Jerne plaque assay (Jerne, 1963, 1974; White, 2010)

Statistics
Body weight, body weight change, and food consumption data were subjected to a parametric one way ANOVA (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1955, 1964) was used to compare the test

substance treated groups to the control group.
The positive control data were evaluated using the Student's t-Test (Sokal and Rohlf, 1981) and compared to the basal diet control group.

Organ weight (wet spleen and thymus), final body weight, and AFC data obtained were first tested for homogeneity of variances using the Bartlett's Chi Square test (Bartlett, 1937). Homogeneous data were evaluated using a parametric one-way ANOVA (Kruskal and Wallis, 1952). When significant differences occurred, the treatment groups were compared to the basal diet control group using Dunnett's test (Dunnett, 1955, 1964). Non-homogeneous data were evaluated using a non-parametric ANOVA (Wilson, 1956). When significant differences occurred, the treatment groups were compared to the basal diet control group using the Gehan-Wilcoxon test when appropriate (Gross & Clark, 1975). The Jonckheere's test (Hollander & Wolfe, 1973) was used to test for dose-related trends across the basal diet control and test substance treated groups. The positive control data were evaluated using the Student's t Test (Sokal & Rohlf, 1981) and compared to the basal diet control group. The criteria for accepting the results of the positive control group included a statistically significant ($p \leq 0.05$) decrease in the response when compared to the response of the basal diet control group.
The AFC data were expressed as Specific Activity, IgM antibody forming cells per million spleen cells (AFC/106 spleen cells), and as IgM Total Spleen Activity (AFC/spleen).
For the purpose of data interpretation, statistical significance was not considered to automatically imply immunotoxicological significance. Conversely, the absence of a statistically significant comparison was not considered solely to imply the lack of a biologically relevant effect.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

The achieved concentrations of glyphosate in the dietary preparation were in the range of 85.6 – 97.5% of nominal, and therefore within the acceptable range of 85 – 115%. The diet formulations were homogeneous and stable for 10 days when stored at room temperature with the following exception. During homogeneity/concentration acceptability testing, the 450 ppm diet formulation was 83.1% of target. The 5500-ppm diet formulation was within acceptable range (90.8%) but was considered low, therefore, calibration standards were prepared as matrix-based samples and a cross-validation was conducted. The diet formulations were reanalysed using matrix-based calibration standards and met the testing facilities SOP acceptance criteria for homogeneity and concentration acceptability. Based on these results, the protocol-specified doses of test substance were offered to the animals. The test substance was not detected in the basal diet that was offered to the basal diet control (Group 1) and positive control (Group 5) groups.

B. MORTALITY AND CLINICAL SIGNS

There were no mortalities observed during the study period.

C. CLINICAL OBSERVATIONS

There were no test substance-related clinical findings.

D. BODY WEIGHT

There were no test substance related effects on body weights in any of the dose groups.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no test substance-related effects on food consumption noted.

The group mean achieved doses are summarised below.

Table B.6.8.2.1-1: Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice ([redacted], 2012): Group mean achieved dose levels of glyphosate

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
1 (vehicle control)*	0	0
2 (low)	500	150.1
3 (mid)	1500	449.1

4 (high)	5000	1447.5
5 (positive control)	50 mg/kg CPS**	-

* basal diet group
** CPS = cyclophosphamide

F. NECROPSY

Gross pathology
There were no test substance-related macroscopic effects.

Treatment with the positive control CPS produced a small thymus in three of the 10 animals. These changes were consistent with the known effects of CPS in female B6C3F1 mice.

Organ weights
There were no test substance-related effects on terminal body weights or on spleen or thymus weights (absolute or relative to final body weight) when the test substance-treated groups were compared to the basal diet control group.

Treatment with the positive control CPS produced statistically significantly lower spleen and thymus weights (absolute and relative to final body weight) when compared to the basal diet control group. These changes were consistent with the known effects of CPS in female B6C3F1 mice.
The results of final body and organ weight determinations are presented in the table below.

Table B.6.8.2.1-2: Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice ([redacted], 2012): Final body weight and organ weight data

Dose group	Body weight (g)[#]	Spleen weight		Thymus	
		Absolut	Relative	Absolut	Relative
		(mg) [#]	(% of body weight) [#]	(mg) [#]	(% of body weight) [#]
1 (vehicle control)*	20.9 ± 0.3	85.3 ± 3.5	0.41 ± 0.02	44.3 ± 3.5	0.21 ± 0.02
2 (low)	20.6 ± 0.2	82.3 ± 4.6	0.40 ± 0.02	41.5 ± 1.9	0.20 ± 0.01
3 (mid)	21.6 ± 0.3	91.6 ± 6.5	0.42 ± 0.03	45.9 ± 2.7	0.21 ± 0.01
4 (high)	21.3 ± 0.2	86.0 ± 3.6	0.40 ± 0.02	42.0 ± 2.6	0.20 ± 0.01
5 (positive control)	21.5 ± 0.3	**50.2 ± 3.2****	**0.23 ± 0.02****	**13.3 ± 0.8****	**0.06 ± 0.01****

[#] Values presented the mean ± SD derived from the number of animals evaluated per dose group
* basal diet group
** Statistically significant from vehicle control at $p \leq 0.01$

G. AFC ASSAY RESULTS
There were no test substance-related effects on spleen cell numbers, and in the functional evaluation of the IgM antibody-forming cell (AFC) response, treatment with glyphosate did not result in a statistically significant suppression of the humoral immune response when evaluated as either Specific Activity (AFC/10^6 spleen cells) or Total Spleen Activity (AFC/spleen). There were no statistically significant differences nor any dose-related trends noted when the basal diet control and test substance-treated groups were compared.

Statistically significantly lower spleen cell numbers, mean specific activity, and mean total spleen activity values were noted in the positive control (CPS treated) group when compared to the basal diet control group. These effects were consistent with the known immunosuppressant effects of CPS and validated the appropriateness of the AFC assay.

The results of the AFC assay are summarised in the table below.

Table B.6.8.2.1-3: Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice ([redacted], 2012): Results of AFC assay

Dose group	Spleen cells (x 10^7)#	IgM AFC / 10^6 spleen cells#	IgM AFC/spleen (x 10^3)#
1 (vehicle control)*	11.29 ± 0.65	1160 ± 131	127 ± 11
2 (low)	11.45 ± 0.64	1273 ± 123	144 ± 16
3 (mid)	13.45 ± 1.24	1368 ± 163	190 ± 37
4 (high)	12.51 ± 0.66	1514 ± 204	195 ± 32
5 (positive control)	**5.18 ± 0.53****	**0 ± 0****	**0 ± 0****

Values presented the mean ± SD derived from the number of animals evaluated per dose group
* basal diet group
** Statistically significant from vehicle control at p ≤0.01

Study conclusion
Repeated dietary administration of glyphosate to females B6C3F1 mice did not suppress the humoral component of the immune system. The no-observed-effect level (NOEL) for suppression of the humoral immune response in female B6C3F1 mice offered glyphosate in the diet for 28 days was considered to be 5000 ppm (equivalent to 1448 mg/kg bw/day), the highest dietary concentration.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Treatment of female B6C3F1 mice for 28 days with glyphosate did not suppress the humoral component of the immune system when evaluated using the AFC assay. The no-observed-effect level (NOEL) was considered to be 5000 ppm (equivalent to 1448 mg/kg of body weight/day) in female mice, the highest dietary concentration. The study was performed according to EPA OPPTS 870.7800 and no deviations were apparent. The study is considered informative and acceptable.

Assessment and conclusion by RMS:
The conclusions made by the applicant are agreed with.

B.6.8.2.2. 8-week oral study of citric acid

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	2010
Report title	An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats
Report No	[redacted]-50361
Document No	Not reported.
Guidelines followed in study	Guideline does not exist. Proof of concept study for salivary gland alterations due to treatment with acidic diet.
Deviations from current test guideline	Not applicable.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes, conducted under GLP.
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a

	Conclusion AGG: The study did not follow a specific guideline but is considered to be fit for purpose to evaluate if salivary gland alterations can be caused by treatment with an acidic diet. The study is therefore concluded to be acceptable.

2. Full summary

A number of repeat dose studies in rodents with glyphosate technical acid have identified alterations of the salivary glands, described as increased basophilic staining and enlargement of cytoplasm, especially in the parotid salivary glands. The toxicological significance of these observations were considered not relevant, by some reviewers and unknown by others. In the 2004 JMPR review of glyphosate, a hypothesis was proposed that the low pH of glyphosate technical acid in the diet caused local irritation in the oral cavity leading to the observed salivary gland effects. The objective of this study was to evaluate the potential effects of low pH diet on the parotid salivary glands. Citric acid was selected as an appropriate surrogate for glyphosate, having both a similar pH-dilution curve and low toxicity. Citric acid was presented in the diet (14000 ppm) and compared with a typical pH basal diet control group. A higher pH diet group fed basal diet with trisodium citrate dihydrate (21400 ppm, an equivalent citrate ion concentration to the citric acid group) was also compared with the typical pH basal diet control group. In addition, low pH aqueous citric acid was administered by gavage and compared to a control deionised water gavage group to evaluate potential systemic effects of the citrate ion on the parotid salivary glands. These five test groups, each consisting of 10 male rats, were dosed for eight weeks (minimum of 56 days).
Clinical signs, bodyweight and food consumption were monitored during the study. All animals were subjected to a gross necropsy examination and a comprehensive histopathological evaluation of tissues was performed. The findings are summarised as follows:
There were no test substance-related clinical signs of toxicity, as well as no test substance-related effects on body weight, and food consumption.

Test substance-related effects on organ weights consisted of statistically significantly higher parotid salivary gland weights in the low pH diet group only (citric acid) when compared to the respective control group. Non-statistically significantly higher parotid salivary gland weights were noted in the gavage citric acid and high pH dietary (trisodium citrate dihydrate) groups when compared to their respective control group. There were no statistically significant test substance-related effects on the fused mandibular/sublingual salivary gland weights when the respective control and test substance-treated groups were compared; however, a non-statistically significantly higher fused mandibular/sublingual salivary gland weight was noted in the low pH diet group (14000 ppm citric acid).

Histological effects consisted of cytoplasmic alterations in the parotid salivary glands characterized by the presence of hypertrophied acinar cells with basophilic granular cytoplasm. Although the overall incidence of affected animals was similar in all control and citric acid or trisodium citrate dihydrate-treated groups, these effects were clearly most severe in the low pH diet group (14000 ppm citric acid in basal diet). With the absence of microscopic findings such as cytotoxicity and hyperplasia, the observed effects are considered to be an adaptive response to local irritation of the low pH diet in the oral cavity rather than an adverse effect.

Citric acid administered orally via gavage or diet and trisodium citrate dihydrate administered via the diet to Sprague Dawley rats for 56 days resulted in higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands in all dose groups (gavage citric acid, diet citric acid, and diet trisodium citrate dihydrate). The magnitude of change in parotid gland weight and severity of the cytoplasmic alteration in the parotid salivary glands was most severe in the low pH diet citric acid group.

I. MATERIALS AND METHODS

A. MATERIALS

Test materials:

Identification: Anhydrous Citric Acid

Description:	White powder
Lot/Batch #:	XR3050
Purity:	99.9%
Stability of test compound:	Stable at room temperature until 2010-01-06
Identification:	Trisodium Citrate Dihydrate (TCD)
Description:	White crystalline solid
Lot/Batch #:	1387609
Purity:	99.3%
Stability of test compound:	Stable at room temperature until 2011-03-01
Vehicle and/ or positive control:	Gavage: deionised water, Diet: plain diet
Test animals:	
Species:	Rats
Strain:	Sprague-Dawley (Crl:CD)
Source:	[redacted]
Age:	Approx. 6 weeks upon beginning of treatment
Sex:	Males
Weight at dosing:	177 - 227 g
Acclimation period:	14 days
Diet/Food:	Certified Rodent LabDiet #5002 (PMI Nutrition International, LLC), *ad libitum* except for fasting period prior to necropsy
Water:	Tap water, *ad libitum*
Housing:	Upon arrival, animals were housed three per cage for approximately 3 days. Thereafter, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 50 ± 20% Air changes: at least 10/hour Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-02-10 to 2009-04-21

Animal assignment and treatment

In an 8 week gavage and feeding study groups of 10 male Sprague Dawley rats received the respective vehicles or test substances for 56 consecutive days via oral gavage (Groups 1 and 3) or in the diet (Groups 2, 4 and 5; see table below). A low pH diet containing 14000 ppm of citric acid in basal diet was offered continuously to Group 4. A high pH diet containing 21400 ppm of trisodium citrate dihydrate in basal diet (at an equivalent citrate ion concentration to Group 4) was offered continuously to Group 5. A concurrent control group (Group 2) received the basal diet on a comparable regimen. Citric acid in the vehicle, deionised water, was administered orally by gavage at a dose level of 791-1316 mg/kg bw/day to Group 3.

Concentrations of the Group 3 formulations were calculated and adjusted weekly, based on the average food consumption and body weights of the Group 4 animals from the previous week of dosing in order to maintain approximately equivalent citric acid dose levels to Group 4. A concurrent gavage control group (Group 1) received the vehicle on a comparable regimen.

Table B.6.8.2.2-1: An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats ([redacted], 2010): Study group assignment

Group number	Test substance application	Dose level (mg/kg bw/day or ppm)	Dose volume (mL/kg)	Number of animals
1	Gavage Vehicle	0	10	10
2	Basal Diet	0	na	10
3	Gavage Citric Acid (low pH)	791-1316	10	10
4	Diet Citric Acid (low pH)	14000 ppm*	na	10
5	Diet Trisodium Citrate (high pH)	21400 ppm*	na	10

na - not applicable, * stoichiometrically equivalent for citrate ion concentration

Observations
All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed once daily for all dietary group animals and twice daily for all oral gavage-dosed animals. Detailed physical examinations were performed weekly.

Body weight
Individual body weights were recorded weekly.

Food consumption and compound intake
Food consumption was recorded weekly.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: parotid salivary glands, mandibular salivary glands and sublingual salivary glands. The mandibular and sublingual salivary glands were weighed together as one organ since they were fused and could not be adequately separated for weighing.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum and femur (incl. joint)), brain (cerebrum at two levels; cerebellum with medulla/pons), caecum, colon, duodenum, epididymides, eyes with optic nerves, gross lesions, harderian glands, heart, ileum, jejunum, kidneys, lacrimal gland (exorbital), liver, lungs (incl. bronchi), mammary gland, lymph nodes (mandibular, mesenteric and axillary), nasal cavity, oesophagus, pancreas, Peyer's patches, pituitary, prostrate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea and urinary bladder.
Microscopic examination was performed on the parotid salivary glands and gross lesions from all animals at the scheduled necropsy.

Statistics
All statistical tests were performed using the [redacted] Toxicology Data Management System (WTDMS™). Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group to its respective control group.
Body weight, body weight change, food consumption, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA identified statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare each of the test substance-treated groups to the respective control group (Group 1 to Group 3 and Group 2 to Groups 4 and 5). Group 1 was also compared to Group 2.
Statistical analysis of the severity of histological changes was conducted. Individual animals were assigned severity scores based on parotid salivary gland changes (0=without histological change, 1=minimal change, 2=mild change, and 3=moderate change). The severity scores were then compared statistically using the Mann-Whitney U-test by comparing Group 1 to Group 3 and Group 2 to Groups 4 and 5.

II. RESULTS AND DISCUSSION

The analyzed citric acid gavage dosing formulations were found to contain the protocol-specified concentration of test article according to specifications in the [redacted] Standard Operating Procedures (85% to 115% of target concentration), were homogeneous, and were stable when refrigerated for 15 days.

The analyzed citric acid (Group 4) and trisodium citrate dihydrate (Group 5) diet admix formulations were found to contain the protocol-specified concentration of test article according to specifications in the WIL Standard Operating Procedures (85% to 115% of target concentration), were homogeneous, and were stable at room temperature for 15 days.

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
All clinical findings in the test substance-treated groups were noted with similar incidence in the control groups, were limited to single animals, and/or were common findings for laboratory rats of this age and strain.

C. BODY WEIGHT
There were no statistically significant differences when the respective control and test substance-treated groups were compared.

D. FOOD AND TEST SUBSTANCE CONSUMPTION
Food consumption was unaffected by citric acid or trisodium citrate dihydrate administration. A statistically significant decrease in food consumption of the gavage citric acid group (Group 3, Week 7/8) was of minor magnitude (-5%), probably due to biological variability and not considered related to test substance administration.

Test item intake in the 14000 ppm citric acid group was 1040 mg/kg bw/day while the intake for the 21400 ppm trisodium citrate dihydrate group was 1607 mg/kg bw/day.

E. PATHOLOGY

Organ weights
Test substance-related effects on organ weights consisted of statistically significant higher absolute and relative parotid salivary gland weights in the low pH diet group (14000 ppm citric acid) when compared to the dietary control group; the magnitude of change was >40% (see table below).
Higher absolute and relative parotid salivary gland weights were also observed in the low pH gavage group (791-1316 mg/kg bw/day citric acid) and in the high pH diet group (21400 ppm TCD) when compared to their respective control groups. However, the parotid salivary gland weight differences in the low pH gavage and high pH diet groups were not statistically significant and were of much lesser magnitude of change.
There were no other statistically significant test substance-related effects on the fused mandibular/ sublingual or parotid salivary gland weights when the control groups and test substance-treated groups were compared.

Table B.6.8.2.2-2: An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats ([redacted], 2010): Mean absolute and relative organ weights (mean ± SD)

	Gavage administration		**Dietary administration**		
	Aqueous control	**791-1316 mg/kg bw/day citric acid**	**Basal diet control**	**Low pH diet, 14000 ppm citric acid**	**High pH diet, 21400 ppm trisodium citrate dihydrate**
Absolute mandibular / sublingual fused glands weight (g)	0.7625 ± 0.05446	0.7873 ± 0.08397	0.7682 ± 0.08670	0.8872 ± 0.16548	0.7869 ± 0.07028

Relative mandibular / sublingual fused glands weight (g/100 g bw)	0.179 ± 0.0105	0.180 ± 0.0178	0.173 ± 0.0221	0.199 ± 0.0339	0.183 ± 0.0201
Absolute parotid gland weight (g)	0.3500 ± 0.12450	0.4082 ± 0.11990	0.2758 ± 0.08514	**0.3905* ± 0.10920**	0.3502 ± 0.08986
Relative parotid gland weight (g/100 g bw)	0.083 ± 0.0299	0.095 0.0304	0.062 ± 0.0194	**0.088* ± 0.0236**	0.082 0.0220

* statistically significantly different from relevant control group ($p<0.05$) using Dunnett's test

Necropsy

All macroscopic findings noted were considered spontaneous and/or incidental in nature and unrelated to test substance administration.

Histopathology

Test substance-related histological effects consisted of a higher severity of cytoplasmic alterations in the parotid salivary glands of the citric acid and trisodium citrate dihydrate-treated groups when compared to their respective control groups (see table below). The severity of cytoplasmic alteration was increased in all dose groups; however, the cytoplasmic alteration was clearly most severe in the low pH diet group (Group 4; 14000 ppm citric acid). Furthermore, statistical significance for the grade of severity was only achieved in the low pH diet group, while the severity in the other dose groups was not statistically significantly different from the respective control group.

Cytoplasmic alteration in the parotid salivary glands was characterized by the presence of hypertrophied acinar cells with basophilic granular cytoplasm. The severity grades varied from minimal to moderate, displayed by increasing numbers of affected acinar cells and more pronounced hypertrophy of acinar cells with increasing severity grade. A minimal severity grade corresponded to the multifocal presence of affected acinar cells in scattered salivary gland lobules. With severity grades of mild or moderate, affected areas, while still considered multifocal, were often coalescing and included affected acinar cells in most salivary gland lobules. Increased numbers of acinar cells were affected, and hypertrophy of acinar cells was more pronounced with increasing severity grade.

Cytotoxicity and hyperplasia were not observed and consequently, the observed changes were considered adaptive responses rather than adverse effects. There were no other test substance-related histological changes.

Table B.6.8.2.2-3: An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats ([redacted], 2010): Histological findings

	Gavage administration		**Dietary administration**		
	Aqueous control	**791-1316 mg/kg bw/day citric acid**	**Basal diet control**	**Low pH diet, 14,000 ppm citric acid**	**High pH diet, 21,400 ppm trisodium citrate dihydrate**
Parotid salivary glands [a]	9	10	10	10	10
Incidence (%)	*100*	*100*	*70*	*100*	*90*
minimal	8	6	5	0	4
mild	1	3	2	6	5
moderate	0	1	0	4	0
Average severity [b]	**1.1**	**1.5**	**0.9**	**2.4****	**1.4**

[a] number of tissues examined from each group

[b] 1= minimal, 2= mild and 3= moderate; animals without a histological change were assigned a severity score of 0

** significantly different from relevant control group ($p<0.01$) using the Mann-Whitney U-Test

Study conclusion

Citric acid administered orally via gavage or diet and trisodium citrate dihydrate administered via the diet to Sprague Dawley rats for 56 days resulted in higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands at all dose levels with equivalent citrate ion doses (791-1316 mg/kg bw/day gavage citric acid, 14000 ppm diet citric acid, and 21400 ppm diet trisodium citrate dihydrate). These effects were noted as most severe in the low pH dietary test group. In the absence of cytotoxicity and hyperplasia the noted effects are considered an adaptive response rather than an adverse effect and are consistent with the hypothesis that low pH diets result in adaptive cellular responses within the salivary glands.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Higher parotid salivary gland weights and histological alterations were noted as statistically significant in the low pH citric acid diet administered to Sprague Dawley rats. This proof of concept study is considered supportive evidence that low pH diets result in adaptive salivary gland alterations, as noted in some glyphosate acid repeat dose dietary studies.

Assessment and conclusion by RMS:
The study did not evaluate glyphosate, but evaluated the effect of acidic diet on salivary glands. The study is considered to be fit for purpose. The effects observed in this study resemble those that were observed in some of the studies with glyphosate. It does however, not exclude the possibility of other mode of actions being behind the observed effects.

This conclusion is in line with the previous EU evaluation (RAR, 2015).

B.6.8.2.3. 28-day oral study, comparison of salivary gland effect in different rat strains

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat
Report No	[redacted]/P/5160
Document No	Not reported.
Guidelines followed in study	No guideline exists for this kind of study.
Deviations from current test guideline	Not applicable.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes, conducted under GLP.
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: The study did not follow a specific guideline but is considered to be fit for purpose to compare the sensitivity of different rat strains to the salivary gland effects.

2. Full summary

The purpose of this study was to investigate the rat strain susceptibility of the effects of glyphosate acid on the salivary gland after 4 weeks administration in these strains of rat. In studies with F344 rats, glyphosate acid has been shown to cause effects on the salivary gland (NTP, 1992[6]). In contrast, there was no evidence of microscopic changes in the salivary gland in a previously conducted 28 day feeding study with glyphosate acid (20000 ppm in the diet) in Alpk:AP_fSD rats, although there was an effect on gland weight [redacted], 1995)[7].

Study groups of 24 male Alpk:AP_fSD (Wistar-derived; AP), Sprague-Dawley (Charles River CD; CD) and Fischer 344 (F344) rats received 0 or 20000 ppm glyphosate acid. Eight animals from each group were killed on Day 29 and the remaining animals were retained without treatment for a further 4 (8 rats/group) or 13 weeks (8 rats/group). Clinical observations, bodyweights and food consumption were measured and at the end of the scheduled periods, the animals were killed and subjected to a necropsy. Salivary glands were weighed and taken for subsequent histopathology examination.

Treatment with 20000 ppm glyphosate acid produced significant reductions in bodyweight and minor reductions in food consumption in AP and CD rats but no effects on bodyweight or food consumption were seen in the F344 rat. Salivary gland weight was unaffected in the CD rat but was increased in both AP and F344 rats at the end of the administration period. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slight effects involving small foci of cells only occurred in the AP and CD strains.
Recovery of effects was apparent in all strains during the recovery periods. Bodyweight and food consumption returned to control values in both AP and CD strains. After four weeks on control diet, significant recovery of the salivary gland changes, in terms of both weight and histopathology, was evident in the F344 strain and the AP and CD rats were indistinguishable from their corresponding controls. After 13 weeks on control diet slightly more treated F344 rats showed minor focal changes in the salivary gland compared to the contemporaneous controls and group mean salivary gland weights were increased slightly.
Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination of the salivary glands showed the most pronounced effect in the F344 strain. Similar but slighter effects occurred in the AP and CD strains.
Complete recovery of effects were apparent in AP and CD strains following the 4-week recovery period and significant recovery had occurred in the F344 strain. It is not clear whether the slightly higher incidence of minor focal changes in the salivary glands of the F344 strain after 13-week recovery was a residual effect of treatment or represented the random variation in the background incidence in this strain.

I. MATERIALS AND METHODS

A. MATERIALS

Test materials:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%
Stability of test compound:	No data given in the report
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Rats

[6] NTP (1992). Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. United States Department of Health and Human Services, National Toxicology Program Toxicity Reports Series Number 16

[7] [redacted] (1995) 28day dietary toxicity study in the rat. [redacted]/L/6624

Strain 1:	Alpk:AP_fSD
Source:	[redacted]
Weight at dosing:	175.0 – 176.1 g
Strain 2:	Sprague-Dawley
Source:	[redacted]
Weight at dosing:	179.6 – 181.5 g
Strain 3:	Fischer 344
Source:	[redacted]
Weight at dosing:	107.4 – 108.9 g
Age:	approx. 28-30 days (on delivery)
Sex:	Males
Acclimation period:	11-13 days
Diet/Food:	CT1 diet (supplied by Special Diet Services Limited, Witham, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed by strain and four per cage.
Environmental conditions:	Temperature: 21 ± 3 °C Humidity: 50 ± 20% Air changes: at least 15/hour Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1996-01-15 to 1996-05-14

Animal assignment and treatment
In a 28 days feeding study groups of 24 male Alpk:AP_fSD (Wistar-derived; AP), Sprague-Dawley (Charles River CD; CD) and Fischer 344 (F344) rats received 0 or 20000 ppm glyphosate acid. Eight animals from each group were killed on Day 29 and the remaining animals were retained without treatment for a further 4 (8 rats/group) or 13 weeks (8 rats/group).
Two test diet batches were prepared prior to start of treatment by mixing 1255 g test substance to 58.745 kg diet and blending. Samples of both preparations were analysed to verify the achieved concentration.

Clinical observations
Cage-side observations were performed daily. A detailed physical examination was performed prior to administration and weekly thereafter. Detailed clinical examinations were performed at the same time as body weights were recorded.

Body weight
Individual body weights were recorded on start of administration and weekly thereafter.

Food consumption and compound intake
Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage.

Sacrifice and pathology
All animals were killed by halothane anaesthesia and subjected to a gross examination of the salivary glands. The salivary glands were weighed (left and right separately) and microscopically examined (left) in animals surviving until the end of the study.

Statistics
All data were evaluated using analysis of variance and/or covariance by the GLM procedure in SAS (1989).

Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based in the error mean square in the analysis.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

The mean achieved concentration of glyphosate acid in both batches of diet was within 2% of the target concentration (see table below).

Table B.6.8.2.3-1: Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat ([redacted], 1996): Achieved concentrations of glyphosate acid in the diet

	Nominal concentration (ppm)	Mean analysed concentration (ppm)	% of nominal concentration
Batch 1	20,000	19,985	99.9
Batch 2	20,000	20,355	101.8

B. MORTALITY

There were no treatment-related deaths. One treated AP rat was killed in Week 7 following accidental damage to its snout.

C. CLINICAL OBSERVATIONS

There were no treatment-related findings in any of the groups noted during the study period.

D. BODY WEIGHT

AP rats

During the administration period significantly lower group mean bodyweight than for control was seen. At the end of the administration period the difference was approximately 7%. The reduction in bodyweight was maintained during the 4-week recovery period (approximately 7% at the end of Week 9) but no differences in bodyweight were apparent by the end of the 13-week recovery period.

CD rats

Group mean bodyweights for treated animals were significantly reduced during the administration period in comparison to controls. The reduction in bodyweight was approximately 7% (after adjusting for initial bodyweight) at the end of the administration period. However, bodyweights quickly recovered and were approximately 4% lower (not statistically significant, after adjusting for initial body weight), compared with control, after the 4-week recovery period, and 5% higher than controls (after adjusting for initial body weight) by the end of the 13-week recovery period.

F344 rats

No treatment related effects were observed.

Table B.6.8.2.3-2: Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat ([redacted], 1996): Body weight effects

	AP		CD		F344	
	0	20000 ppm	0	20000 ppm	0	20000 ppm
Body weight (g)						
Week 1	176.1	175.0	181.5	179.6	107.4	108.9
Week 2	228.9	**222.0****	240.7	**228.4****	132.8	133.2
Week 3	280.3	**270.4****	291.7	**273.3****	157.8	158.5
Week 4	319.5	**304.2****	331.0	**306.0****	181.7	180.8
Week 5	357.4	**331.0****	366.5	**337.1****	205.8	203.3

Week 6	382.4	**355.4****	384.6	**360.7***	217.4	216.8
Week 7	413.0	**381.8****	410.9	389.0	233.4	233.1
Week 8	432.0	**399.1****	425.3	407.1	244.6	244.9
Week 9	455.1	**422.5****	446.4	427.9	257.9	259.5
Week 10	449.0	434.3	442.5	446.6	270.0	260.9
Week 11	464.6	452.9	459.1	466.1	282.6	272.6
Week 12	479.9	467.4	471.9	482.0	291.5	281.8
Week 13	491.5	481.4	483.4	496.1	301.9	289.9
Week 14	498.8	491.6	489.1	505.9	307.3	297.4
Week 15	503.9	496.6	495.9	515.1	31.6	303.6
Week 16	508.8	504.7	500.9	523.0	321.8	311.3
Week 17	516.9	513.0	509.3	533.1	329.6	318.9
Week 18	523.1	518.9	514.1	534.4	336.0	325.5

E. FOOD CONSUMPTION

AP rats
Overall, food consumption in the treated group tended to be slightly lower than the control during the administration period although this achieved statistical significance only in Week 7. No effects were seen at the end of the recovery period.

CD rats
Group mean food consumption for treated animals was generally lower than controls during the administration period, reaching statistical significance in Week 2 and 4. Food consumption for the recovery animals returned to control levels by Week 8.

F344 rats
There was no evidence of any treatment related effects.

F. NECROPSY

Organ weights
There was no evidence of any effects of glyphosate acid on the salivary gland weight at any time point in CD rats. On the contrary, salivary gland weights were increased in the treated AP and F344 rats at the end of the administration period in comparison to control. While no effects were noted in the 4- or 13-week recovery AP animals, in F344 rats the salivary gland weights were still increased at these time points, although there was clear evidence of recovery.

Table B.6.8.2.3-3: Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat ([redacted], 1996): Mean salivary gland weights at necropsy

Organ	AP		CD		F344	
	0	**20000 ppm**	**0**	**20000 ppm**	**0**	**20000 ppm**
Terminal weight (g)						
Left salivary gland	0.652	**0.740***	0.715	0.695	0.461	**0.666****
Right salivary gland	0.523	**0.659***	0.623	0.626	0.422	**0.577***
Weight after 4 week recovery (g)						
Left salivary gland	0.748	0.703	0.844	0.742	0.488	0.555
Right salivary gland	0.639	0.623	0.701	0.637	0.428	**0.505***
Weight after 13 week recovery (g)						
Left salivary gland	0.750	0.760	0.790	0.819	0.623	0.612
Right salivary gland	0.669	0.681	0.668	0.705	0.485	0.528

* Statistically significant difference from the control group mean at p<0.05(Student's t-test, two-sided).
** Statistically significant difference from the control group mean at p<0.01 (Student's t-test, two-sided).
n = 8 animals/study group

Macroscopic findings
No macroscopic abnormalities were seen in salivary glands in any rat, either at the end of the administration period or after the 4- or 13-week recovery periods.

Microscopic findings
Treatment-related findings were confined to the parotid salivary gland and comprised alteration in the staining of the cytoplasm of the acinar cells. The affected cells appeared strongly basophilic and enlarged (recorded as basophilia of parotid acinar cells).

At the end of the four-week administration period, this change was most prominent in F344 rats. All rats showed marked cytoplasmic basophilia that was diffuse, involving the whole of the parotid gland. However, no evidence of cell degeneration or necrosis was seen. Most of the control F344 rats also showed a minor degree of basophilia involving occasional acinar cells only.
The other two strains, AP and CD, both showed the same effect in the parotid gland after four weeks of treatment, but at a much reduced severity compared to the F344. In addition, the distribution was different in that only small focal groups of acinar cells were affected in the AP and CD rats in contrast to the diffuse involvement seen in the F344. The effect was weakest in the CD rat.

The incidence data at the end of the administration period indicate that the background change varies in control rats in the three strains. None was seen in the AP controls, there was a single CD control rat with a minimal focal change, whereas 7 out of 8 F344 controls showed minor changes.
After four weeks recovery in the F344 strain the severity of the parotid basophilia was reduced to minimal or slight and affected small foci of acinar cells only. No changes were seen in the CD rats and only a single AP rat showed a minimal change. As an AP control rat showed changes at this time point this is considered not to be related to treatment.
After 13 weeks recovery no treatment-related changes were seen in the AP and CD strains. Slightly more of the F344 rats showed minor focal changes compared to the corresponding control group but the study author concluded this may reflect variations in the background spontaneous change rather than a residual effect of treatment.

Table B.6.8.2.3-4: Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat ([redacted], 1996): Histopathological findings in salivary glands

Finding	AP		CD		F344	
	0	20000 ppm	0	20000 ppm	0	20000 ppm
Termination*						
Atrophy (marked)	0 / 8	0 / 8	1/8	0 / 8	0 / 8	0 / 8
Interstitial fibrosis (marked)	0 / 8	0 / 8	1/8	0 / 8	0 / 8	0 / 8
Basophilia of parotid acinar cells (minimal/slight/moderate/marked)	0 / 8	8 / 8 (1/6/1/0)	1/8 (1/0/0/0)	7 / 8 (4/1/2/0)	7 / 8 (6/1/0/0)	8 / 8 (0/0/0/8)
Microscopic findings after 4 week recovery*						
Mononuclear cell infiltration (minimal)	0 / 8	1 / 8	0 / 8	0 / 8	1 / 8	0 / 8
Basophilia of parotid acinar cells (Minimal/slight)	1 / 8 (1/0)	1 / 8 (1/0)	0 / 8	0 / 8	0 / 8	6 / 8 (5/1)
Mucous metaplasia of parotid (slight)	0 / 8	1 / 8	0 / 8	0 / 8	0 / 8	0 / 8
Microscopic findings after 13 week recovery*						
Mononuclear cell infiltration (minimal)	0 / 8	0 / 7	0 / 8	0 / 8	1 / 8	1 / 8
Atrophy (minimal)	0 / 8	0 / 7	0 / 8	0 / 8	1 / 8	0 / 8
Basophilia of parotid acinar cells (minimal/slight/moderate)	1 / 8 (1/0/0)	1 / 7 (1/0/0)	1 / 8 (1/0/0)	1 / 8 (1/0/0)	1 / 8 (1/0/0)	5 / 8 (2/2/1)

* number of animals affected / total number of animals examined

Study conclusion

Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slighter effects occurred in the AP and CD strains involving small foci of cells only.
Complete recovery of effects was apparent in AP and CD strains following the 4-week recovery period and significant recovery had occurred in the F344 strain. It is not clear whether the slightly higher incidence of minor focal changes in the salivary glands of the F344 strain after 13-week recovery was a residual effect of treatment or represented the random variation in the background incidence in this strain.

Assessment and conclusion by applicant:
Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slighter effects occurred in the AP and CD strains involving small foci of cells only. A complete recovery from the effects on the salivary gland was observed in the AP and CD strains following a 4-week recovery period. A significant, though incomplete, recovery had occurred in the F344 strain, supporting the hypothesis that low pH diets induce a non-adverse adaptive response in salivary glands. Following a 13-week recovery period a slightly higher incidence of minor focal changes in the salivary glands of the F344 strain treatment group was noted, compared with the F344 strain control group and compared with the AP and CD strain. The study is considered acceptable.

Assessment and conclusion by RMS:

The study showed clear strain differences in the effect on the salivary gland. However, this conclusion does not significantly impact the evaluation of glyphosate as it does not show that the effect observed with the most sensitive strain (F344) would also not occur in humans.

This conclusion is in line with the previous EU evaluation (RAR, 2015).

B.6.8.2.4. In vivo pharmacology screening in rat and guinea pig

Data point:	CA 5.8.2/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate Technical: Pharmacology Screening Study in the Rat
Report No	434/021
Document No	Not reported.
Guidelines followed in study	JMAFF, 59 Nohsan No. 4200 (1985)
Deviations from current test guideline	Not applicable.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes, conducted under GLP.
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: Some limitations were noted. The stability of the test material was not reported and the dose level tested was not analytically verified. Therefore, the study is considered to be acceptable but with restrictions (reliable with restrictions).

Full summary

The test material was evaluated for evidence of pharmacological activity using a series of *in vivo* and *ex vivo* screening methods. For *in vivo* studies five male and five female rats were dosed with glyphosate technical at a dose level of 5000 mg/kg with similar sized control groups receiving vehicle only. Approximately one hour after dosing control and treated animals were examined for either haematological changes, electrocardiographic changes or behavioural/functional changes. *Ex vivo* studies on the isolated guinea pig ileum and isolated rat gastrocnemius muscle were performed using saturated solutions of the test material.

***In vivo* studies**
There were no differences in response between treated and control animals.

***Ex vivo* studies**
Glyphosate Technical (12 mg/mL) caused a contractile response to isolated guinea pig ileum similar to that seen with acetylcholine. The effect seen was abolished when the ileum was pre-incubated with atropine sulphate.
Injection of tubocurarine resulted in a significant diminution of the contractile response of the rat gastrocnemius muscle when the sciatic nerve was stimulated. On the contrast, there was no effect on muscle contraction when either glyphosate technical or physiological saline was injected.

Conclusion
At a maximum oral gavage dose level of 5000 mg glyphosate technical/kg bw there were no effects seen from the *in vivo* screens performed. When administered to the isolated guinea pig ileum glyphosate technical caused a contractile response similar to that seen with known parasympathomimetic agents. Evaluation of innervated muscle response showed that glyphosate technical, when administered at the maximum solubility concentration in physiological saline, did not cause any neuromuscular blocking activity.

I. MATERIALS AND METHODS

A. MATERIALS

Test materials:

Identification:	Glyphosate Technical	
Description:	White powder	
Lot/Batch #:	H95D161A	
Purity:	95.3%	
Stability of test compound:	No data given in the report.	
Vehicle and/ or positive control:	*in-vivo*	1% carboxymethyl cellulose
	ex-vivo (guinea pig)	distilled water, krebs physiological buffer solution,
	ex-vivo (guinea pig)	physiological saline

Test animals:

in-vivo	Species:	Rats
Strain:		Sprague-Dawley (CD)
Source:		[redacted]
Age:		no data
Sex:		Males and females
Weight at dosing:		176 - 200 g
Acclimation period:		At least 6 days
Diet/Food:		SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*

Water:		Tap water, *ad libitum*
Housing:		By sex in groups of five in polypropylene cages with stainless steel grid floors.
Environmental conditions:		Temperature: 19 – 25 °C Humidity: 40 – 75% Air changes: at least 15/hour Photocycle: 12 hours light/dark cycle
ex-vivo	Species:	Guinea pig
Strain:		Dunkin Hartley
Source:		[redacted]
Age:		no data
Sex:		Males
Weight at dosing:		250 - 300 g
Acclimation period:		no data
Diet/Food:		Guinea Pig FDI Diet (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
Water:		Tap water, *ad libitum*
Housing:		By sex in groups of up to three in polypropylene cages with solid floors and sawdust bedding.
Environmental conditions:		Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: at least 15/hour Photocycle: 12 hours light/dark cycle
in-vivo	Species:	Rats
Strain:		Sprague-Dawley (CD)
Source:		[redacted]
Age:		no data
Sex:		Males and females
Weight at dosing:		110 - 125 g
Acclimation period:		no data
Diet/Food:		SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
Water:		Tap water, *ad libitum*
Housing:		By sex in groups of five in polypropylene cages with stainless steel grid floors.
Environmental conditions:		Temperature: 19 – 25 °C Humidity: 30 – 70% Air changes: at least 15/hour Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

Test material preparation

In vivo

The test material was suspended daily in 1% carboxymethyl cellulose (vehicle) by weighing an amount into a suitable container and adding vehicle to make the appropriate final volume. Homogeneity was assured by mixing the formulations with a Silverson mixer/homogeniser.

Ex vivo – Guinea pig ileum

The test material was dissolved initially in distilled water and subsequently in freshly prepared Krebs physiological buffer solution. A saturated solution was used. The test material/buffer solution was shaken until

the test material was dissolved.

Ex vivo – Rat gastrocnemius muscle
The test material was dissolved in sterile physiological saline. A saturated solution was used. The test material/vehicle was shaken until the test material was dissolved.

The test material was dissolved at the maximum solubility of 12 g/L for all *ex vivo* studies.

Methods for *in vivo* studies
Dose rationale
The selected dose level (5000 mg/kg bw) was based on the results of an acute oral toxicity study in rats previously conducted by the laboratory.

Group allocation and dosing
Animals were dosed by oral gavage administration once only on the day allocated for each dose group as determined by the type of evaluation specified.

Table B.6.8.2.4-1: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Study design

Group	Males	Females	Evaluation	Dose (mg/kg bw)	Dose volume (mL/kg bw)
1	5	5	Haematology	0*	10
2	5	5		5000	10
3	5	5	Electrocardiography	0*	10
4	5	5		5000	10
5	5	5	Behaviour/functional tests	0*	10
6	5	5		5000	10

*Control animals were treated with vehicle only.

Haematology
Approximately one hour after dosing blood samples were taken via a marginal tail vein from all animals from Groups 1 and 2. The blood was collected into tubes containing Potassium EDTA anticoagulant. For measurement of clotting (prothrombin) time samples were collected into tubes containing sodium citrate (0.11 mol/L).

The following parameters were evaluated: Haemoglobin (Hb), Total erythrocyte count (RBC), Haematocrit (Hct), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (WBC), Platelet Count (PIT), Clotting (Prothrombin) time (CT).

Electrocardiography (ECG)
Approximately one hour after dosing animals from Groups 3 and 4 were anaesthetised using halothane anaesthesia and assessed for cardiac activity using an electrocardiograph. A limb lead was attached to each limb and connected to the electrocardiogram. The equipment was set to lead II measurement at a sensitivity of either 10 mm/mvolt or 5 mm/mvolt and a chart speed of 25 mm/second. Alternative control and treated animals were evaluated.

The following parameters are measured: Heart rate, P-R interval, QRS interval, Q-T interval, P-amplitude, R-amplitude, and T-amplitude.

Behavioural/functional tests
Approximately one hour after dosing animals from Groups 5 and 6 were placed individually in a purpose built arena and assessed for behaviour and response to various stimuli using a modified Irwin Screen.

The following observations were performed: salivation, hypo/hyperthermia, skin colour, respiration, lacrimation, palpebral closure, piloerection, exophthalmia, gait, twitches, tremors, convulsions, abnormal behaviour, tail elevation, transfer arousal, urination count, defaecation count and vocalisation count.

The following manipulative tests were performed: Finger Approach, Touch Escape, Tail Pinch, Toe Pinch, Grasp Response, Auditory Startle Response, Pupil Response to Light and Palpebral Reflex.

Methods for *ex vivo* studies

Evaluation of isolated guinea pig ileum
Sections of ileum were dissected from previously untreated guinea pigs killed by cervical dislocation. The sections of ileum were transferred to a purpose-built isolated organ bath containing Krebs buffer solution. The isolated ileum was connected to the lever arm of an isotonic transducer by a cotton ligature. The transducer was connected to a chart recorder. Contractions of the isolated ileum could then be recorded. Standard solutions of acetylcholine, a known agonist, were prepared and added to the volume of buffer solution used to bathe the isolated ileum. A maximum volume of 2 mL was used for all experiments to ensure the integrity of the tissue in the medium. The contraction response of isolated ileum was recorded for each concentration of acetylcholine to produce a standard curve. Between the addition of each new concentration of acetylcholine, the buffer in the organ bath was flushed out and replaced by fresh buffer. The test material, dissolved in buffer, was added and its response compared with standards.
Following initial results, an antagonist (atropine) to the effects of acetylcholine was added together with the agonist. The results were then compared with the effects of only an antagonist added to the test material.

The following were recorded: response to acetylcholine (agonist, sympathomimetic), response to test material, response to atropine (antagonist) and acetylcholine (agonist), and response to atropine (antagonist) and test material.

Evaluation of rat gastrocnemius muscle
Previously untreated rats were killed by cervical dislocation. The abdomen was immediately dissected open and the dorsal aorta exposed. A butterfly needle was inserted into the dorsal aorta, near to the bifurcation in a posterior direction.
A volume of 0.3 mL of lithium heparin at a concentration of 10 mg/mL in sterile saline was injected into the dorsal aorta followed by 0.5 mL of sterile saline. The gastrocnemius muscle of the hind limb was exposed with the sciatic nerve intact. The gastrocnemius muscle was detached from the ankle joint and this area was ligated with cotton, which was then attached to the lever arm of a transducer. The limb was held in place by a series of pins. An electrical stimulus of 12 volts was applied to the sciatic nerve and the muscle response was recorded. This action was repeated at approximately twelve—second intervals until sufficient responses had been recorded.
The experiment was repeated on separate animals with doses of tubocurarine (positive control) injected into the dorsal aorta instead of sterile saline. The experiment was also repeated on a separate animal with the test material dissolved in sterile saline.

The following were recorded: Response to injection of sterile saline, response to injection of tubocurarine, and response to injection of test material.

Evaluation of data

In vivo
Data were processed to give group mean values and standard deviations where appropriate.

Evaluation of electrocardiographic outputs
All measurements were performed using a vernier calliper with digital readout. All measurements were recorded twice in order to minimise operator bias.

Isolated guinea pig ileum
The final concentration of test material in the organ bath was based on the serial dilution value plus the additional dilution in the total volume of the organ bath (50 mL).

II. RESULTS AND DISCUSSION

In vivo studies

Evaluation of blood parameters
There were no biologically significant differences, among the parameters measured, between treated and control animals.

Table B.6.8.2.4-2: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Group mean haematology values

Parameter	0 mg/kg bw		5000 mg/kg bw	
	Male (n=5)	Female (n=5)	Male (n=5)	Female (n=5)
WBC	14.2 ±3.2	11.0 ±2.2	12.8 ±3.9	10.6 ±2.6
RBC	6.8 ±0.3	6.8 ±0.3	6.6 ±0.3	6.8 ±0.3
HB	14.2 ±0.3	14.1 ±0.4	14.8 ±0.6	14.6 ±0.4
HCT	41.9 ±1.0	41.6 ±0.9	42.5 ±1.1	42.1 ±1.9
MCV	61.8 ±2.4	61.6 ±2.7	64.0 ±2.5	61.5 ±1.4
MCH	20.9 ±0.9	20.8 ±1.0	22.2 ±0.8	21.4 ±0.6
MCHC	33.9 ±0.5	33.8 ±0.9	34.8 ±0.6	34.7 ±0.6
PLT	1079.2 ±169.9	1020.0 ±55.7	1024.8 ±89.5	1164.0 ±160.9
Clotting Time (seconds)	28.8 ±1.7	26.2 ±1.3	27.3 ±1.2	26.3 ±2.2

Evaluation of cardiovascular system
One female of the control group had cardiac arrhythmia characterised by extra systoles. There were no biologically significant differences in the mean values recorded during electrocardiography (ECG) for wave amplitudes, wave intervals or heart rate.

Table B.6.8.2.4-3: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Group mean ECG values

Group Sex	Dose		Amplitude (mv)			Interval (msec)			Heart rate (bpm)
			P	R	T	P-R	QRS	Q-T	
3 male	0 mg/kg bw	**Mean**	**0.09**	**0.70**	**0.08**	**43.20**	**16.60**	**32.68**	**408.0**
		SD	0.02	0.23	0.09	6.32	7.77	10.37	22.6
		N	5	5	5	5	5	5	5
3 female		**Mean**	**0.09**	**0.59**	**0.06**	**49.20**	**15.00**	**35.70**	**441.0**
		SD	0.03	0.13	0.02	4.41	5.91	12.28	34.4
		N	4	4	4	4	4	4	4
4 male	5000 mg/kg bw	**Mean**	**0.12**	**0.68**	**0.11**	**37.20**	**17.84**	**31.00**	**441.6**
		SD	0.05	0.19	0.06	8.62	7.50	11.95	20.2
		N	5	5	5	5	5	5	5
4 female		**Mean**	**0.10**	**0.75**	**0.09**	**37.08**	**17.00**	**35.36**	**415.2**
		SD	0.02	0.13	0.03	9.22	2.33	5.16	34.5
		N	5	5	5	5	5	5	5

bpm = beats per minute, SD = standard deviation

Behavioural and functional tests
There were no biologically significant differences in behaviour and response to manipulative tests. The intergroup variances in response were considered to be within normal variation for this model.

Table B.6.8.2.4-4: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Group mean summary of behavioural assessments*

Parameter	0 mg/kg bw													5000 mg/kg bw											
	Male (n=5)							Female (n=5)							Male (n=5)							Female (n=5)						
	Number of animals classified as (score)																								
	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6
Transfer arousal					5							5					1		4			1				4		
Tail elevation			5							5							5							4		1		

Finger approach				4		1				5				2		3					4		1
Touch escape			1		1		3				1		4				4		1			3		2
Tail pinch			1		4										2		3							
Grasp response			4		1			2		1		2		1		2		2			2		1	
Vocalisation	4		1					2	2			1		4			1				2	2	1	
Toe pinch				2		3											1		4					

Comment of assessor: only parameters with values differing between treated and control groups listed: no differences between treated and control groups were seen for all other behavioural/functional parameters that had been assessed. Scores 0-6 were not clearly explained in study report, however higher scores were indicative for higher grade of activity.

Ex vivo studies

Evaluation of isolated guinea pig ileum

The addition of acetylcholine to the medium containing the isolated guinea pig ileum resulted in contraction of the tissue in a concentration—related response. Incubation with atropine sulphate immediately prior to addition of acetylcholine reduced or stopped the contraction response in a concentration related manner.

The addition of Glyphosate Technical at the maximum solubility in buffer also resulted in contraction of the ileum. The force of contraction was increased by an increasing volume of the test material in solution. Incubation with atropine sulphate prior to addition of Glyphosate Technical also resulted in the lack of a contractile response.

Table B.6.8.2.4-5: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Response of guinea pig ileum to a known agonist and glyphosate

Agonist		**Height of response (mm)**	**% of maximum response**
Test material	**Final concentration**		
Acetylcholine bromide	2.2×10^{-6} m	39	100
	1.8×10^{-6} m	33.5	85.9
	1.5×10^{-6} m	25.5	65.4
	1.0×10^{-6} m	15	38.5
Glyphosate (in distilled water)	4.26×10^{-4} m	7	17.9
	7.1×10^{-4} m	19	48.7
Glyphosate (in Krebs buffer)	7.1×10^{-4} m	12	30.8
	1.42×10^{-3} m	11	28.2
	2.84×10^{-3} m	24.5	62.8

Table B.6.8.2.4-6: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Response of guinea pig ileum to a known agonist and glyphosate in the presence of a known antagonist

Agonist		**Antagonist**		**% of maximum response**
Test material	**Final concentration (M)**	**Test material**	**Final concentration (M)**	
Acetylcholine bromide	3.0×10^{-6}	---		118.8
				108.2
				100
	1.5×10^{-6}			54.1
	1.0×10^{-6}			47.1
	4.0×10^{-7}			12.9
	2.0×10^{-7}			8.2
	3.0×10^{-6}	Atropine sulphate	1.0×10^{-8}	0
			5.0×10^{-9}	18.8
			2.0×10^{-9}	76.5
	2.0×10^{-6}		2.0×10^{-9}	30.6
Glyphosate	2.84×10^{-3}	---		62.4
				41.2
	2.84×10^{-3}	Atropine sulphate	4.0×10^{-9}	0
				0

Table B.6.8.2.4-7: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Response of guinea pig ileum to a known agonist and glyphosate in the presence of a known antagonist

Agonist		Antagonist		% of maximum response
Test material	**Final concentration (M)**	**Test material**	**Final concentration (M)**	
Acetylcholine bromide	2.0×10^{-6}	---		62.1
	3.0×10^{-6}			63.8
				70.0
				79.3
				100
	3.0×10^{-6}	Atropine sulphate	4.0×10^{-9}	0
			2.0×10^{-9}	0
Glyphosate	1.42×10^{-3}	---		13.8
	2.84×10^{-3}			44.8
	2.84×10^{-3}	Atropine sulphate	2.0×10^{-9}	3.4
				0
			4.0×10^{-9}	0

Evaluation of rat gastrocnemius muscle

Injection of tubocurarine, a known neuromuscular blocking agent, at a concentration of 25 mg/mL resulted in a significant reduction of the contractile response of the rat gastrocnemius muscle when the sciatic nerve was stimulated. There was no effect on muscle contraction when either Glyphosate Technical (12 mg/mL) or physiological saline was injected. The difference in force of response seen with Glyphosate Technical and physiological saline can be attributed to individual animal variation.

Table B.6.8.2.4-8: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Comparison of force of contraction of innervated skeletal muscle (gastrocnemius)

Test material	Concentration	Range of response in mm (n = 5)	Average response (mm)	% of control
Physiological saline		21 - 28	24.8	100
Tubocurarine	25 mg/mL	3 - 6	4.6	18.5
Glyphosate Technical	12 mg/mL	23 - 38	32.4	130.6

Study conclusion

At a maximum dose level of 5000 mg/kg bw glyphosate technical no effects were seen in the *in vivo* screens performed. When administered to the isolated guinea pig ileum *ex vivo*, glyphosate technical concentrations at millimolar (mM) levels caused a contractile response similar to that seen with a known parasympathomimetic agent at micromolar (µM) levels.

Assessment and conclusion by applicant:

In vivo

At a maximum dose level of 5000 mg glyphosate technical/kg bw no biologically relevant effects on the treated animals were seen in the *in vivo* screens performed. During extensive clinical observations parameters indicative for a disturbance of the animal's health status, such as hypo/hyperthermia, skin colour, lacrimation, respiration, palpebral closure, and salivation were similar between vehicle control and treated rats. This confirmed that the selected dose level had no toxic effect on the rats. The intergroup variances in response to manipulative tests can be considered as normal biological variation.

Similarly, there were no relevant differences between treated and control animals in the haematological and cardiac parameters measured.

Ex vivo: Muscle contractibility
Evaluation of innervated rat gastrocnemius muscle response showed that glyphosate, when administered at the maximum solubility concentration in physiological saline, did not cause any neuromuscular blocking activity. The effect of glyphosate technical was directly compared to that of a known neuromuscular blocking agent (atropine sulphate), which caused a significant reduction in the muscle response to electrical stimulation of the sciatic nerve.

Ex vivo: Intestinal contractibility
Addition of acetylcholine as a positive control substance to isolated guinea pig ileum resulted in contraction in a concentration-dependent response at micromolar (µM) concentrations. Atropine sulphate immediately prior to addition of acetylcholine reduced or stopped the contraction response in a concentration related manner. This confirmed the reliability of the methodology. Glyphosate caused a contractile response of isolated guinea pig ileum at much higher millimolar (mM) concentrations, which could be antagonized with atropine. Thus, a parasympathomimetic activity of glyphosate was observed in this *ex vivo* model at high concentrations.

The study used appropriate negative control animals, as well as appropriate positive control substances (agonists and antagonists). The age of study animals was not stated. The study is considered acceptable.

Assessment and conclusion by RMS:
No OECD test guideline is available for this type of study. Some limitations were noted. The stability of the test material was not reported and the dose level tested was not analytically verified. Therefore, the study is considered to be acceptable but with restrictions (reliable with restrictions).

The conclusions made by the applicant are agreed with. The *in vivo* study does not give an indication of a pharmacological effect on the parameters evaluated. In the *ex vivo* study glyphosate did not cause neuromuscular blocking activity up to the highest concentration tested. In the *ex vivo* study on intestinal contractibility glyphosate did induce a contractile response. However, this effect occurred only at fairly high concentrations (12 mg/L) and therefore the *in vivo* relevance of this finding is questionable.
This conclusion is in line with the previous EU evaluation (RAR, 2015). It was noted in the previous review that the results seem to be a bit contradictory to the adrenergic mechanism postulated by Chan and Mahler (1992) for salivary gland effects, but it must be taken into account that different tissues were investigated.

B.6.8.2.5. In vivo pharmacology study in ICR mice and albino rabbits

Data point:	CA 5.8.2/005
Report author	[redacted]
Report year	1992
Report title	Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study
Report No	[redacted] 90-0149/[redacted]-92-15
Document No	Not reported.
Guidelines followed in study	None stated.
Deviations from current test guideline	Not applicable.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes.
Acceptability/Reliability:	Conclusion GRG: Supportive, Category 2a Conclusion AGG: No OECD test guideline is available for this type of study. Some limitations were noted. The stability of the test material was not reported and the dose level tested was not analytically verified.

	Therefore, the study is considered to be acceptable but with restrictions (reliable with restrictions).

Full summary

Effects of a single intraperitoneal (mice) or single intravenous (rabbits) administration of the ammonium salt of glyphosate (purity: 94.78%) on clinical appearance, and nervous, respiratory and circulatory system were assessed. Groups of three male and female ICR mice (15 male and 15 female in total) received 78, 313, 1250, or 5000 mg glyphosate/kg bw intraperitoneally. Groups of three male rabbits (30 male in total) received 7.81, 31.3, 125 or 500 mg glyphosate/kg bw intravenously. Mice and half of the male rabbits were clinically observed at frequent intervals after dosing for up to seven days. The other half of male rabbits was used for digital monitoring of respiratory and circulatory parameters under urethane-anaesthesia.

At the top dose levels, all mice died within 0.5 hours and all anesthetized rabbits within a few minutes after injection. Non-anesthetized rabbits survived intravenous application of 500 mg/kg bw (dose resulting from highest water soluble concentration) although animals showed decrease in spontaneous activity and increase in respiratory rate. The rabbits completely recovered within 3 hours. In rabbits, which died, heart rate, blood pressure and respiratory rate decreased and voltage of QRS complex in electrocardiogram were noted. At the next lower dose levels (1250 mg/kg bw in mice or 125 mg/kg bw and 31.3 mg/kg bw in rabbits, respectively), transient symptoms like a decrease in blood pressure, reduced activity and neuromuscular signs were observed but cleared to normal values or behaviour within some hours at the latest. No abnormalities were observed in mice dosed at 313 mg/kg bw or less and in rabbits dosed at 7.81 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:	Ammonium salt of glyphosate (MON-8750)
Identification:	Not stated
Description:	Not stated
Manufacturer	Not stated
Lot/Batch #:	RUD-9201-3544F
Purity:	94.78 %
Storage conditions:	Sealed container in a refrigerator
Stability of test compound:	Not stated
Vehicle:	Physiological saline (0.9 % NaCl), pH 5.0

Test animals:

Species:	Mouse
Strain:	ICR (SPF)
Source:	[redacted]
Age:	7 weeks
Sex:	Male/female
Weight at dosing:	Males: 32 - 41 g, females : 26 - 33 g
Acclimation period:	At least 7 days
Diet/Food:	Certified pelleted diet MF (Oriental Yeast Co., Ltd., Tokyo), *ad libitum*
Water:	Water from a private well passed through precipitating and sedimentary procedures and sterilized by ultraviolet light and chlorine, *ad libitum*
Housing:	Three mice per aluminium cage

Environmental conditions:	Temperature: 22 ± 1 °C Humidity: 55 ± 5 % Air changes: 15/hour (all fresh air system) Photocycle: 12 hours light/dark cycle
Test animals:	
Species:	Rabbit
Strain:	Japanese white strain (SPF)
Source:	[redacted]
Age:	10 weeks
Sex:	Males
Weight at dosing:	2.3 - 2.8 kg (clinical observation group) 2.4 - 3.0 kg (respiration, blood pressure, ECG group)
Acclimation period:	At least 7 days
Diet/Food:	Certified pelleted diet RC4 (Oriental Yeast Co., Ltd., Tokyo), *ad libitum*
Water:	Same as for mice
Housing:	Individually in aluminium cage
Environmental conditions:	Same as for mice

B. STUDY DESIGN

Table B.6.8.2.5-1: Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study ([redacted], 1992): Animal assignment and treatment

Parameter investigated	**Species**	**Sex**	**Route**	**Volume (mL/kg bw)**	**Number of animals per dose**				
					Intraperitoneal dose (mg/kg bw)				
					0	78.1	313	1250	5000
Clinical observation	Mouse	Male	Intraperitoneal	20	3	3	3	3	3
		Female			3	3	3	3	3
					Intravenous dose (mg/kg bw)				
					0	7.81	31.3	125	500 *
Clinical observation	Rabbit	Male	Intravenous	2	3	3	3	3	3
Respiration, blood pressure, ECG					3	3	3	3	3

ECG = electrocardiogram
Control groups received physiological saline at the same volume of administration
* highest soluble concentration for intravenous route

The test substance was dissolved in physiological saline (0.9 % NaCl) adjusting the pH to ca 5.0 by NaOH solution. Intraperitoneal and intravenous routes were chosen to achieve complete absorption of the test substance.

Clinical Observation in mice
Neuropharmacological changes of male and female mice were examined according to Irwin's method (Irwin 1967, in Animal and Clinical Pharmacologic Techniques in Drug Evaluation, Nodine and Siegler). Animals were observed prior to, and 5 min., 0.5, 1, 3, and 6 hours after dosing and then once a day for a week.

Clinical Observation in rabbits

Detailed observations were conducted according to the information/reference provided in the study report. Animals were observed prior to, and 5 min., 0.5, 1, 3, and 6 hours after dosing and then once a day for a week.

Monitoring of respiratory and circulatory parameters in rabbits
Three rabbits per dose were anesthetized with urethane (ca. 1.5 g/kg bw subcutaneous). Rabbits were hold in dorsal position. All parameters were recorded on a polygraph (RM-6000, Nihon Koden Inc., Tokyo). A homeothermic blanket (KN-474, Natsume Seisakusho Co., Ltd.) was used to keep the body temperature constant. The test substance was administered to a rabbit after steady recordings were obtained. Recordings were continued for 4 hours after dosing.

A thermistor type respiration sensor (TR-612T, Nihon Koden Inc.) was fixed to a cannula inserted into a cut opening of the trachea. Respiratory rate was measured by a digital counter (AT-601G, Nihon Koden Inc.). Blood pressure was measured via a cannula inserted into the left femoral artery connected to a high pressure transducer (TP-200T, Nihon Koden Inc.). Systolic, diastolic and mean blood pressures were measured by a digital counter (AP-611G, Nihon Kohden Inc.). ECG was recorded via electrodes fixed to the extremities by Lead II. Heart rate was measured from the pulse of blood pressure by a digital counter (AT-601G, Nihon Kohden Inc.)

Statistics
Student's t-test was used to compare a dosed group with the corresponding control group. Differences between groups were considered to be statistically significant at the $p \leq 0.05$ level.

II. RESULTS AND DISCUSSION

A. CLINICAL OBSERVATIONS IN MICE
All mice of the top dose died within 0.5 h after application. Clinical signs observed after application consisted of manifestation of passivity, a decrease in spontaneous activity, reactivity, and pain response, clonic and tonic convulsions, a decrease in righting reflex, limb tone, body tone, abdominal tone, grip strength, corneal reflex and ipsilateral flexor reflex (IFR). In addition a decrease in alertness, abnormal posture (prone position) and abnormal skin color (cyanosis) was observed in male mice. Staggering gait was observed for females of the high dose.
At the mid dose group of 1250 mg/kg bw abnormal behaviour was observed in male and female mice for awareness, motor activity, central nervous system (CNS), excitation, posture, motor incoordination, muscle tone, reflexes, and autonomic signs. Animals recovered within1 hour after administration.
No clinical signs were observed at the doses of 313 mg/kg bw or less.

Table B.6.8.2.5-2: Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study ([redacted], 1992): Summary of in vivo experiments in mice

Test			Intraperitoneal dose* (mg/kg bw)			
			78.1	**313**	**1250**	**5000**
Clinical observation	Male	Dead/dosed	0/3	0/3	0/3	3/3
		Abnormal signs	–	–	+	++
	Female	Dead/dosed	0/3	0/3	0/3	3/3
		Abnormal signs	–	±	+	++

* Three mice per sex and per dose were treated with a single intraperitoneal injection)
Rating: (–) no, (±) possible, (+) slight, (++) moderate, (+++) severe effect

B. CLINICAL OBSERVATIONS IN RABBITS
Rabbits in the 500 mg/kg bw dose group showed a decrease in spontaneous activity and an increase in respiratory rate within 0.5 hours after injection and completely recovered within 3 hours. No distinct abnormalities were observed at the doses of 125 mg/kg bw or less.

C. RESPIRATORY AND CIRCULATION SYSTEM IN RABBITS
A transient decrease in blood pressure during the injection was observed in rabbits treated with 31.3, 125 and 500 mg/kg bw. The decrease in blood pressure fully recovered to the control level within a few minutes after the injection at 31.3 and 125 mg/kg bw. Rabbits of the high dose groups showed a decrease in blood pressure, respiratory rate, heart rate, and a voltage of QRS complex in ECG, and died within a few minutes after the

injection. The tidal volume (amplitude of respiration) was augmented after the injection. No treatment related effects were observed at 7.81 mg/kg bw.

Table B.6.8.2.5-3: Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study ([redacted], 1992): Summary of in vivo experiments in rabbits

Test				Intravenous dose* (mg/kg bw)			
				7.81	**31.3**	**125**	**500**
Clinical observation	Male	Non-anesthetized	Dead/dosed	0/3	0/3	0/3	0/3
			Abnormal signs	–	–	±	+
Respiration, Blood pressure and ECG	Male	Anesthetized	Dead/dosed	0/3	0/3	0/3	3/3
			Respiratory rate	–	–	–	+ (decrease)
			Blood pressure	–	+ (decrease)	+ (decrease)	++ (decrease
			Heart rate	–	–	–	+ (decrease)
			ECG	–	–	–	+ (decrease in QRS complex)

* Three male rabbits were dosed for each test group by single intravenous injection)
Rating: (–) no, (±) possible, (+) slight, (++) moderate, (+++) severe effect

Study conclusion
In mice, most of the abnormal sings were manifested at the lethal dose of 5000 mg/kg bw and abnormal signs at the dose of 1250 mg/kg bw disappeared within 3 hrs after administration. In rabbits, intravenous injection of the test substance produced no death at the dose of 500 mg/kg bw, which is a practically maximum dose based on its water solubility. These facts suggest that human risk after an incidental exposure of ammonium salt of glyphosate would be low in the field. It is suggested that the acute toxicity of the test substance is low. Serious hazards could not be expected in humans after an acute exposure of the test substance except for the case receiving an extraordinary large amount of the test substance.

Assessment and conclusion by applicant:
A pharmacological study on the toxicity of the ammonium salt of glyphosate was conducted in mice and male rabbits. The test substance was dissolved in physiological saline and adjusted to pH 5 with sodium hydroxide. Different doses up to a top dose level of 5000 mg/kg bw was administered to mice by single intraperitoneal injection. In rabbits the top dose level administered by single intravenous injection was 500 mg/kg bw, which reflected the highest soluble concentration.

At the top dose levels, all mice died within 0.5 hours and all anesthetized rabbits within a few minutes after injection. Non-anesthetized rabbits survived intravenous application of 500 mg/kg bw. They showed transient clinical abnormalities, but fully recovered within 3 hours. At the next lower dose levels transient symptoms like a decrease in blood pressure, reduced activity and neuromuscular signs were observed but cleared to normal values or behaviour within some hours at the latest. At doses up to 125 mg/kg bw in mice and 7.8 mg/kg bw in rabbits no abnormalities were noted.

Based on the available data it cannot be assessed if there was an interaction with urethane used for anaesthesia and the test substance resulting in the lethality of rabbits dosed at the top dose or whether the surgery together with the high dose resulted in the mortality. The study is however not relevant for classification purposes and does also not need to be considered in human risk assessments.

Assessment and conclusion by RMS:
No OECD test guideline is available for this type of study. Some limitations were noted. The stability of the test material was not reported and the dose level tested was not analytically verified. Therefore, the study is considered to be acceptable but with restrictions (reliable with restrictions).

The assessment and conclusion of the applicant is agreed. Adverse effects were noted such as clinical signs,

decreased respiratory rate, decrease blood pressure and a decrease in QRS complex. However, the effects occurred at high intravenous and intraperitoneal doses and therefore the study does not impact the overall risk assessment of glyphosate.

The study was not evaluated by the RMS in the previous RAR (2015). Instead the study summary of the original DAR was copied where it was concluded that an impact on cardiorespiratory functions is involved in acute toxicity.

B.6.8.2.6. In vivo toxicodynamic study in rats

Data point:	CA 5.8.2/006
Report author	Anonymous
Report year	1988
Report title	Toxicodinamic study of glyphosate in rat
Report No	Not stated
Document No	Not reported.
Guidelines followed in study	None stated.
Deviations from current test guideline	Not applicable.
Previous evaluation	Not accepted in RAR (2015).
GLP/Officially recognised testing facilities	No. (GLP was not compulsory at the time the study was performed; non-guideline, investigative study).
Acceptability/Reliability:	Conclusion GRG: Supportive, Category 3a Conclusion AGG: The study is considered to be acceptable but with restrictions (reliable with restrictions) due to the reporting limitations on the study design.

The toxicodynamics of glyphosate were examined in rats by the measurement of heart rate, ECG, venous- and arterial blood pressure and body temperature.

The administered dose of 5000 mg/kg bw glyphosate, a dose close to the oral LD_{50} in rats according to the literature, killed the animals narcotised with chloralose-urethane mixture within 2 to 7 hours. After treatment, decreased pulse and respiratory rate was observed along with decreased systemic arterial pressure. ECG-II and venous pressure changes were incidental and insignificant. The mean median mercury pressure of the artery carotis was 102 mm Hg before treatment, 73 mm Hg and 54—55 mm Hg one hour and 2-3 hours after treatment, respectively. Body temperature was unchanged due to the applied experimental procedure. White spots were seen in the edges of livers of the dead animals. Microscopically, the spots were free of erythrocytes. When blood samples were haemolysed with distilled water and analysed spectrophotometrically, an absorption maximum of 567 nm was found. The haematin hydrochloride peak was recorded at 680 nm.

I. MATERIALS AND METHODS

A. MATERIALS

Non-labelled test material: Glyphosate

Identification: Technical grade glyphosate

Description: White crystalline substance appearance

Lot/Batch #:	72390788
Purity:	96%
Stability of test compound:	Not stated.

Test animals:

Species:	Rat
Strain:	S/Wistar (SPF breeding stock of LATI)
Source:	Not stated.
Age:	Not stated.
Sex:	Males
Weight at dosing:	>400 g
Acclimation period:	Not stated.
Diet/Food:	Not stated.
Water:	Not stated.
Housing:	Shoebox-type macrolone cages
Environmental conditions:	Temperature: Not stated. Humidity: Not stated. Air changes: Not stated.

B. STUDY DESIGN AND METHODS

The reported acute oral lethal dose of glyphosate is 4900 mg/kg bw in rats. For the present study 5000 mg/kg bw was selected as the dose so that the dose would terminate the animals within a day, and allow sufficient time for observations before death.

Anaesthesia was carried out by IV injection of an aqueous solution containing chloralose-urethane in a 1:5 ratio. Injection volume depended on the degree of anaesthesia. The trachea was prepared by a medium incision on the neck and cannulated. To record the respiratory rate and volume the cannula was connected to a Hellige pulsotachometer.

Blood pressure in the left articular carotis was recorded by an 8-channel Hellige polygraph, supplemented with a Statham transducer-electromanometer, with a selector for continuous systolic diastolic and medium pressure. Venous pressure of the right ventricular jugular at the thoracic level was also recorded by the Statham head electromanometer polygraph. Heparin was administered into the cannula only. Lead ECG-II was recorded continuously by means of electrodes positioned into the paw of the animals. Body temperature was measured by a rectally inserted thermistor tube connected to the polygraph.

After half an hour to allow for stabilisation of the above parameters, animals were treated with 5000 mg/kg bw test compound suspended in an aqueous solution of mucilage hydroxyacetyl cellulose. Recordings on the Hellige polygraph were measured in the following sequence: heart rate, ECG, respiration, venous-, arterial pressure, body temperature. Recordings were made with 0.05 and 50 mm/sec paper speed. However, in case of faster recordings, values corresponding to different time intervals were indicated on the paper by hand.

II. RESULTS AND DISCUSSION

Heart rate

At study initiation the mean heart rate (derived from 10 animals) was 462 ± 40 min^{-1} (a level acceptable in anaesthetised rats). One hour after administration of 5000 mg/kg bw glyphosate the mean pulse rate decreased by about 5 %. The mean pulse rate consisted of both increased and decreased individual values, which were, perhaps, without toxicological significance, as no similar changes were observed at later time intervals.

ECG

No specific lead-II alterations were observed, although elevated ST and R waves, increased QRS, wide and spiked T wave were occasionally seen. No test compound-related, specific abnormalities were detected even before the observed reduction in systemic pressure.

Respiration rate
The pre-treatment value (58/min) was slightly decreased 1 hour after treatment (55/min). Further decreases were seen at later time points: pulse rate at the end of the 2nd hour was 54/min (i.e. 7 % lower than at study initiation).

Venous pressure
Most values were at baseline level (around 0 mmHg), the maximum/minimum alterations were within 5 mmHg.

Arterial pressure
Values at study initiation (mean value 102 mmHg; a range of 80- 130 mmHg) decreased about 30 % after 1 hour (mean value 73 mm Hg). The mean value after 2 hours was 54 mm Hg (i.e. 50 % of study initiation value).

Gross pathology
Dosing was performed faultless in each case. Thoracic and abdominal organs were examined in all animals. Urinary vesicles were full of urine, due to the chloralose anaesthesia. No macroscopic changes were observed in animals surviving for 23 hours. In animals who survived for up to 4 to 5 hours, disseminated white spots of 2 to 5 mm size were seen, the origin of which were not in the gastric wall or intestines. Following microscopic examination, it was evident that the isolated spots were free of erythrocytes.

***In vitro* studies with blood**
Blood was brown when dissolved in physiological saline. When crystallised glyphosate was added to untreated rat blood at room temperature, a discolouration was observed which started after 2 to 3 minutes and resulted in a stable brown colour after 5 minutes. Based on visual assessment, the brown solution was unchanged and without sediment after 24 hours, and recorded a maximum absorption at 657 nm spectrophotometrically. The absorption maximum was 680 nm in the same photometer.

Table B.6.8.2-1: Toxicodinamic study of glyphosate in rat (Anonymous, 1988): Effects of a 5000 mg/kg bw per oral dose of glyphosate on Wistar rats No. 1 to 5

Parameter	Hour 0	Hour 1	Hour 2	Hour 3
Rat No. 1				
Pulse rate (min^{-1})	440	450	450	EXIT
ECG II	Normal	Normal	S-T elevation	-
Respiratory rate (min^{-1})	50	40 – 50	65	-
Venous pressure (mmHg)	0-1	0	0	-
Arterial pressure (mmHg)	105	75	50	-
Body temperature (°C)	37	37	37	-
Rat No. 2				
Pulse rate (min^{-1})	440	460	470	EXIT
ECG II	Normal	Normal	QRS increased	-
Respiratory rate (min^{-1})	70	60	Not measured	-
Venous pressure (mmHg)	-2 - 0	-5 - 0	0	-
Arterial pressure (mmHg)	100	55	30	-
Body temperature (°C)	37	37	37	-
Rat No. 3				
Pulse rate (min^{-1})	460	450	370	EXIT
ECG II	Confused	Normal	Normal	-
Respiratory rate (min^{-1})	40	50	55	-
Venous pressure (mmHg)	-	-	-	-
Arterial pressure (mmHg)	110	70	70	-
Body temperature (°C)	36	37	37	-
Rat No. 4				
Pulse rate (min^{-1})	430	460	EXIT	-
ECG II	Normal	Wedged	-	-

Respiratory rate (min^{-1})	52	63	-	-
Venous pressure (mmHg)	0 - 1	-1 - 0	-	-
Arterial pressure (mmHg)	105	105	-	-
Body temperature (°C)	36	36	-	-
Rat No. 5				
Pulse rate (min^{-1})	510	450	450	450
ECG II	Normal	Normal	Normal	Normal
Respiratory rate (min^{-1})	65	40	30	40
Venous pressure (mmHg)	2	2	2	2
Arterial pressure (mmHg)	100	70	50	40
Body temperature (°C)	37	36	37	37

Table B.6.8.2-2: Toxicodinamic study of glyphosate in rat (Anonymous, 1988): Effects of a 5000 mg/kg bw per oral dose of glyphosate on Wistar rat No. 6

Parameter	**Hour 0**	**Hour 1**	**Hour 2.5**	**Hour 4.5**	**Hour 7**
Pulse rate (min^{-1})	480	450	430	450	450
ECG II	Normal	Normal	Normal	Normal	Normal
Respiratory rate (min^{-1})	50	60	40	65	67
Venous pressure (mmHg)	1	7	3	2	0
Arterial pressure (mmHg)	110	90	50	100	60
Body temperature (°C)	36	36	38	36	36

Table B.6.8.2-3: Toxicodinamic study of glyphosate in rat (Anonymous, 1988): Effects of a 5000 mg/kg bw per oral dose of glyphosate on Wistar rats No. 7 to 10

Parameter	**Hour 0**	**Hour 1**	**Hour 2**	**Hour 3**	**Hour 4**
Rat Nº 7					
Pulse rate (min^{-1})	420	390	420	EXIT	
ECG II	Normal	Normal	R high top flat	-	-
Respiratory rate (min^{-1})	65	50	75	-	-
Venous pressure (mmHg)	1	1	1	-	-
Arterial pressure (mmHg)	75	60	55	-	-
Body temperature (°C)	35	35	36	-	-
Rat No. 8					
Pulse rate (min^{-1})	420	360	450	EXIT	-
ECG II	Normal	Normal	Normal	-	-
Respiratory rate (min^{-1})	56	50	75	-	-
Venous pressure (mmHg)	0 - 1	-1 - 0	-1 - 0	-	
Arterial pressure (mmHg)	130	75	50	-	
Body temperature (°C)	36	37	37	-	
Rat No. 9					
Pulse rate (min^{-1})	540	480	-	480	EXIT
ECG II	Normal	Normal	-	Normal	-
Respiratory rate (min^{-1})	70	55	-	55	-
Venous pressure (mmHg)	1	-1 - 0	-	-1 - 0	-
Arterial pressure (mmHg)	80	45	-	45	-
Body temperature (°C)	36	35	-	35	-
Rat No. 10					
Pulse rate (min^{-1})	480	480	480	EXIT	-
ECG II	Normal	Normal	Normal	-	-
Respiratory rate (min^{-1})	60	60	55	-	-
Venous pressure (mmHg)	0 - 1	0	0	-	-
Arterial pressure (mmHg)	105	80	70	-	-

Body temperature (°C)	36	36	36	-	-

Assessment and conclusion by applicant:
Half an hour after anesthetising with a chloralose-urethane mixture, male Wistar rats were administered a single dose of 5000 mg glyphosate/kg bw by oral gavage. All animals died within 2 to 7 hours. Treatment was followed by a marked decrease in arterial pressure by approximately 50 % two hours after treatment as compared to the initial values. There was no clear impact on heart rate and respiratory rate. ECG and venous pressure changes, if occurring in some animals, were considered incidental and body temperature was not affected.

While this study does provide some useful information, there are serious reporting deficiencies and errors within the report. Therefore, the study is only considered supportive.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. Under the current study conditions there is no adverse effect on heart rate, ECG, respiration rate, venous pressure, arterial pressure and body temperature after exposure to glyphosate.
According to the applicant, the study was not accepted in the previous evaluation (RAR, 2015). The RMS, however, was not able to retrieve this study from the previous evaluation and therefore the study is considered as newly submitted.

B.6.8.2.7. In vivo single oral dose study with glyphosate and 2,4-D, isoproturon or metolachlor

Data point:	CA 5.8.2/007
Report author	[redacted]
Report year	1988
Report title	Synergism and potentiation in rats of glyphosate (technical) of [redacted]
Report No	Not stated
Document No	Not stated
Guidelines followed in study	None stated.
Deviations from current test guideline	Not applicable. No purity or batch number of test materials given. Total dosing volume for combined tests unclear.
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; non-guideline, investigative study).
Acceptability/Reliability:	Conclusion GRG: Supportive, Category 2a Conclusion AGG: The study investigates the effects of glyphosate in combination with either 2,4-D sodium salt, isoproturon or metolachlor. No batch number, stability or purity of the test material is reported. The study provides little relevant information for the renewal evaluation of glyphosate and is considered supplementary.

Full summary

Groups of 10 male Wistar rats were administered 5000 mg/kg bw of glyphosate as a single oral dose at a constant dose volume of 10 mL/kg bw in corn oil. Three compounds were administered in combination with 5000 mg glyphosate per kg bw: a) 2,4-D sodium salt (at six dose intervals between 380 and 1200 mg/kg bw), b)

isoproturon (at five dose intervals between 3200 and 5000 mg/kg bw), and c) metolachlor (at six dose intervals between 2100 and 3800 mg/kg bw). After simultaneous dosing, the rats were observed for 14 days for toxic symptoms and mortality.

Prior to simultaneous dosing, each of the three compounds were administered to groups of rats individually to measure toxic symptoms and mortality.

LD_{50} values determined for the three substances, 2,4-D sodium salt, isoproturon and metalochlor were 750, 3900, and 2700 mg/kg bw, respectively. When administered after previous administration of 5000 mg glyphosate/kg bw, LD_{50} values of the three substances were reduced to 585, 3600, and 2450 mg/kg bw, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate (technical)
Description:	White powder
Lot/Batch #:	Not stated
Purity:	Not stated
Stability of test compound:	Not stated
Other materials	a) 2-4 D, sodium salt (white powder) b) Isoproturon (white powder) c) Metolachlor (liquid)
Vehicle:	Corn oil (dose volume: 10.0 mL/kg bw)

Test animals:

Species:	Albino rat
Strain:	Wistar
Source:	[redacted]
Age:	Not stated
Sex:	Male
Weight at dosing:	130 to 160 g
Acclimation period:	Not stated
Diet/Food:	Pelleted feed (supplied by Lipton India Ltd., Bangalore), overnight fasting.
Water:	Water, *ad libitum*
Housing:	Five per cage (poly propylene cages) with husk bedding
Environmental conditions:	Temperature: 19–25 °C Humidity: 25—75 % Air changes: not reported Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

The test material in different doses was administered orally by gavage to groups of 10 male rats and the animals were observed for 14 days for toxic symptoms and mortality. LD_{50} was calculated by the method of Litchfield & Wilcoxon (1949). LD_{50} was determined for each of the compounds; 2-4 D, sodium salt, isoproturon and metolachlor.

After determining the LD_{50} of each test material groups of 10 rats received a single administration of glyphosate

followed by 2-4 D, sodium salt, isoproturon and metolachlor. The post administration observation period was 14 days.

II. RESULTS AND DISCUSSION

A. GLYPHOSATE IN COMBINATION WITH 2-4 D, SODIUM SALT

Prior to administration in combination with glyphosate, the LD_{50} of 2-4 D, sodium salt was determined. Six groups, all containing 10 rats, were administered 2-4 D, sodium salt (380, 470, 600, 750, 940, and 1200 mg/kg bw). Reported symptoms included; salivation, ataxia, and loss of righting reflex. The LD_{50} with fiducial limits was calculated: 750 (656 to 858) mg/kg bw.

Table B.6.8.2.7-1: Synergism and potentiation in rats of glyphosate (technical) of [redacted] ([redacted], 1988): Mortality chart of 2-4 D, sodium salt

Number of rats	Dose (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	380	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	470	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	600	0	2	0	0	0	0	0	0	0	0	0	0	0	0	20
10	750	1	4	0	0	0	0	0	0	0	0	0	0	0	0	50
10	940	3	3	2	0	0	0	0	0	0	0	0	0	0	0	80
10	1200	4	6	-	-	-	-	-	-	-	-	-	-	-	-	100

Glyphosate (5000 mg/kg bw) was then administered in combination with 2-4 D, sodium salt (380, 470, 600, 750, 940, and 1200 mg/kg bw) to six groups all containing 10 rats. Mortalities are tabulated below. Reported symptoms included; salivation, ataxia, and loss of righting reflex. The LD_{50} of 2-4 D, sodium salt in the presence of glyphosate was calculated: 585 mg/kg bw.

Table B.6.8.2.7-2: Synergism and potentiation in rats of glyphosate (technical) of [redacted] ([redacted], 1988): Mortality chart for different doses of 2-4 D, sodium salt combined with glyphosate

Number of rats	Glyphosate + 2-4 D, sodium salt (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	5000 + 380	0	1	0	0	0	0	0	0	0	0	0	0	0	0	10
10	5000 + 470	1	2	0	0	0	0	0	0	0	0	0	0	0	0	30
10	5000 + 600	2	3	0	0	0	0	0	0	0	0	0	0	0	0	50
10	5000 + 750	4	4	0	0	0	0	0	0	0	0	0	0	0	0	80
10	5000 + 940	5	4	1	-	-	-	-	-	-	-	-	-	-	-	100
10	5000 + 1200	7	3	-	-	-	-	-	-	-	-	-	-	-	-	100

B. GLYPHOSATE IN COMBINATION WITH ISOPROTURON

Prior to administration in combination with glyphosate, the LD_{50} of isoproturon was determined. Five groups, all containing 10 rats, were administered isoproturon (3200, 3600, 4000, 4500, and 5000 mg/kg bw). No symptoms were recorded. The LD_{50} with fiducial limits was calculated: 3900 (3582 to 4247) mg/kg bw.

Table B.6.8.2.7-3: Synergism and potentiation in rats of glyphosate (technical) of [redacted] ([redacted], 1988): Mortality chart for isoproturon

Numbe	Dose	Animals died on day	Mortalit

r of rats	(mg/kg bw)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	y (%)
10	3200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	3600	0	2	1	0	0	0	0	0	0	0	0	0	0	0	30
10	4000	2	3	1	0	0	0	0	0	0	0	0	0	0	0	60
10	4500	2	3	3	0	0	0	0	0	0	0	0	0	0	0	80
10	5000	5	4	1	-	-	-	-	-	-	-	-	-	-	-	100

Glyphosate (5000 mg/kg bw) was then administered in combination with isoproturon (3200, 3600, 4000, 4500, and 5000 mg/kg bw) to five groups all containing 10 rats. Mortalities are tabulated below. Reported symptoms included; profuse salivation, urination and loose motion, ataxia, chromodacryorrhea, muscle paralysis. The LD_{50} of isoproturon in the presence of glyphosate was calculated: 3600 mg/kg bw.

Table B.6.8.2.7-4: Synergism and potentiation in rats of glyphosate (technical) of [redacted] ([redacted] 1988): Mortality chart for different doses of isoproturon combined with glyphosate

Number of rats	Glyphosate + isoproturon (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	5000 + 3200	0	2	0	0	0	0	0	0	0	0	0	0	0	0	20
10	5000 + 3600	2	2	1	0	0	0	0	0	0	0	0	0	0	0	50
10	5000 + 4000	3	5	0	0	0	0	0	0	0	0	0	0	0	0	80
10	5000 + 4500	3	6	1	-	-	-	-	-	-	-	-	-	-	-	100
10	5000 + 5000	5	5	-	-	-	-	-	-	-	-	-	-	-	-	100

C. GLYPHOSATE IN COMBINATION WITH METOLACHLOR

Prior to administration in combination with glyphosate, the LD_{50} of metolachlor was determined. Six groups, all containing 10 rats, were administered Metolachlor (2100, 2400, 2700, 3000, 3400, and 3800 mg/kg bw). Reported symptoms included; sluggishness and hunched back. The LD_{50} with fiducial limits was calculated: 2700 (2437 to 2992) mg/kg bw.

Table B.6.8.2.7-5: Synergism and potentiation in rats of glyphosate (technical) of [redacted] ([redacted], 1988): Mortality chart for metolachlor

Number of rats	Dose (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	2100	0	1	0	0	0	0	0	0	0	0	0	0	0	0	10
10	2400	2	1	0	0	0	0	0	0	0	0	0	0	0	0	30
10	2700	2	2	1	0	0	0	0	0	0	0	0	0	0	0	50
10	3000	1	5	1	0	0	0	0	0	0	0	0	0	0	0	70
10	3400	4	4	1	0	0	0	0	0	0	0	0	0	0	0	90
10	3800	4	6	-	-	-	-	-	-	-	-	-	-	-	-	100

Glyphosate (5000 mg/kg bw) was then administered in combination with metolachlor (2100, 2400, 2700, 3000, 3400, and 3800 mg/kg bw) to six groups all containing 10 rats. Mortalities are tabulated below. Reported symptoms included; sluggishness and hunched back. The LD_{50} of metolachlor in the presence of glyphosate was calculated: 2450 mg/kg bw.

Table B.6.8.2.7-6: Synergism and potentiation in rats of glyphosate (technical) of [redacted] [redacted], 1988): Mortality chart for different doses of metolachlor combined with glyphosate

Number of rats	Glyphosate + metolachlor (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	5000 + 2100	1	1	0	0	0	0	0	0	0	0	0	0	0	0	20
10	5000 + 2400	1	2	1	0	0	0	0	0	0	0	0	0	0	0	40
10	5000 + 2700	3	4	0	0	0	0	0	0	0	0	0	0	0	0	70
10	5000 + 3000	4	4	1	0	0	0	0	0	0	0	0	0	0	0	90
10	5000 + 3400	4	6	-	-	-	-	-	-	-	-	-	-	-	-	100
10	5000 + 3800	3	7	-	-	-	-	-	-	-	-	-	-	-	-	100

Table B.6.8.2.7-7: Synergism and potentiation in rats of glyphosate (technical) of [redacted] ([redacted], 1988): Summary of determined LD_{50} values in male Wistar rats

	2,4-D, Na salt	5000 mg/kg bw glyphosate + 2,4-D, Na salt	Isoproturon	5000 mg/kg bw glyphosate + isoproturon	Metalochlor	5000 mg/kg bw glyphosate + metolachlor
LD_{50} (mg/kg bw)	750	585	3900	3600	2700	2450

Study conclusion

A single oral administration of glyphosate in combination with 2,4-D, sodium salt, isoproturon, or metolachlor caused no potentiation in albino rats.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Groups of 10 male Wistar rats were administered 5000 mg/kg bw of glyphosate as a single oral dose at a constant dose volume of 10 mL/kg bw in corn oil, followed by administration of different doses of three other substances, 2,4-D sodium salt, isoproturon, or metolachlor. The exact total dosing volume of combined administration was not reported.

The LD_{50} values of these three substances decreased when co-administered with glyphosate.

The value of this study is questionable, however, information on combined acute effects might be used as supplementary data.

Assessment and conclusion by RMS:

The study investigates the effects of glyphosate in combination with either 2,4-D sodium salt, isoproturon or metolachlor. The study provides little relevant information for the renewal evaluation of glyphosate since the label does not describe the combined used with other plant protection active substances and is therefore considered supplementary.

The LD50 values of the three substances in combination with glyphosate were slightly lower than when treated alone although the differences were marginal and at times within the confidence interval of the LD50 for the compounds alone.

B.6.8.2.8. In vivo acute oral toxicity in goat

Data point:	CA 5.8.2/008
Report author	[redacted]
Report year	1987
Report title	The acute toxicity of glyphosate in female goats
Report No	80006
Document No	[redacted]-80-450
Guidelines followed in study	None stated.
Deviations from current test guideline	Not applicable, non-guideline study.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, The study is claimed to be in general conformance with GLP practices but no quality assurance was conducted and no GLP certificate for the lab is included in the study report. However, GLP was not yet compulsory at the time of conduct of the study.
Acceptability/Reliability:	Conclusion GRG: Supportive, Category 2a Conclusion AGG: Several limitations were noted. A very wide range of age of animals was used (8 months to 4 years) with a wide range in weight 13.37 - 44 kg. Moreover, environmental conditions during housing was not reported. The dosing volume used seems fairly high (500 ml/goat). One control and one goat in the 4620 mg/kg bw gave birth during the study while one control goat gave birth one hour prior to treatment. It is unclear if other females goats were pregnant during the study. Pneumonia was observed in a number of animals independent of dose indicating that some kind of infection occurred in the animals. The clinical chemistry parameters were compared based on time of death instead of based on dose level. These limitation indicate that the study population was not homogenous. However, it is recognized that no guideline exist for this type of study. Overall, the study is concluded to be supportive

Full summary

Four groups consisting of five female Spanish goats were administered single doses of glyphosate acid via stomach tube at doses of 1980, 3090, 4620, and 10000 mg/kg bw. Water was used as a vehicle to give a constant dosing volume of 500 mL per animal. Four control groups of five goats each were administered water only.

All animals treated with 10000 and 4620 mg/kg bw and one animal treated with 3090 mg/kg bw died. The acute oral LD_{5O} (14 days) was calculated to be 3530 mg/kg bw.

Clinical signs observed in goats that died included cessation of feeding activity, loss in body weight, abdominal distress, depression, ataxia, mild diarrhoea, and, shortly prior to death, recumbency. Pulmonary oedema to some degree was noted in several animals and was judged a terminal event.

Clinical signs observed in surviving goats included decreased feed consumption, diarrhoea, and body weight loss. Clinical signs noted in goats treated with the minimum dose included decreased feed consumption and diarrhoea. All clinical signs were absent at the end of the experiment (14–15 days).

Of the animals that survived until terminal sacrifice, those in the 3090 mg/kg bw group had mean body weights less than their respective control group. Goats in the 1980 mg/kg bw group had mean body weights similar to their respective control group. Feed consumption was not measured, but cessation of feeding activity was observed in all treatment groups.

No gross lesions that could be attributed to treatment were seen.
Histological examination was performed only on heart, liver, kidney, spleen and other tissues with grossly visible lesions from animals given glyphosate at 4620 and 3090 mg/kg bw, and of 4 sacrificed control animals. Mild to severe tubular nephrosis was the most consistent histopathological lesion observed in goats that died. 4/5 of the goats that died during the observation period had mild fatty change in the liver. Animals in the 3090 mg/kg bw group that survived lacked such lesions.

Changes of blood urea nitrogen concentration, serum creatinine concentration, and in the number of circulating segmental neutrophils were the most consistent laboratory findings at all dose levels. However, clinical chemistry parameters for surviving treated animals were similar to those of the control animals towards the end of the study.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	N-(phosphonomethyl)glycine (glyphosate)
Description:	White crystalline solid
Lot/Batch #:	XHJ-64, NBP1494248
Purity:	98.7%
Stability of test compound:	>1 year at 29.5 ºC
Vehicle:	Water was used to suspend the test article and to wash any residue from the container holding the dose and from the stomach tube. Total volume of water used was approximately 500 mL/goat

Test animals:

Species:	Goat
Description:	Spanish goats
Source:	[redacted]
Age:	Approximately 8 months to 4 years of age
Sex:	Females
Weight at dosing:	13.37 - 44 kg (mean bodyweight range 24.4 - 29.5 kg)
Acclimation period:	30 days
Diet/Feed:	During the initial acclimatization (outdoor): bermuda grass hay and commercial goat meal containing not less than 16% crude protein [Purina® Goat Chow® (Coarse) (Ralston Purina Company, Gonzales, TX, USA)]. After the initial acclimatisation (indoor) the goats were fed bermuda grass only.
Water:	Fresh water and mineral salt blocks were available at all times.
Housing:	During acclimatisation: Outdoor covered pens After dosing: Indoor pens
Study initiation:	22 Dec 1980
Study termination	05 May 1981
Environmental conditions:	Not reported

B. STUDY DESIGN AND METHODS

Animal assignment and treatment

Groups of female Spanish goats received the test item, glyphosate, at dose levels of 10000, 4620, 3090, and 1980 mg/kg bw by oral gavage as a single dose in a sequential manner. Initially, the test item was given to a group receiving 10000 mg/kg bw and subsequent doses were selected based on the observed responses. Control animals were treated with tap water. All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14 – 15 days after treatment. Only goats without visible evidence of active disease and weighing more than 13 kg were used in this study.

Concomitant treatments
During the initial holding period, all goats were vaccinated for enterotoxaemia, treated for internal parasites and observed for signs of disease.

Randomization
All goats available for experimental use were ranked according to increasing bodyweight. The resulting ranking was divided into 5 nearly equal groups. Treatment and control groups of 5 goats each were formed by randomly selecting 1 goat from each of the 5 divisions of the ranking in order that the resulting groups would be representative with respect to bodyweight of the pool from which they were selected.

Body weight
Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14.

Sampling time points
Blood samples were collected on 3 separate days during pre-treatment and after dosing (designated as day 0) on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Clinical biochemistry
Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) and allowed to clot at ambient temperature; the serum was separated by centrifugation. Serum lactic dehydrogenase activity, creatine phosphokinase activity, and glucose concentration was determined as soon as possible after sample collection.
An atomic absorption spectrophotometer (Perkin Elmer Model 403, Perkin-Elmer Corp.) was used to determine serum levels of calcium, magnesium, potassium, and sodium as outlined in the manufacturer's manual. Serum levels of glucose, urea nitrogen, total protein, alkaline phosphatase, lactic dehydrogenase, and glutamic oxaloacetic transaminase were determined on automated equipment (Gilford model 3500 computer directed analyser, Gilford Instruments, Inc.) according to manufacturer's procedures. Four other serum components were determined with the same automated equipment; however, different procedures and reagents were used for these determinations, i.e., inorganic phosphorous (Inorganic Phosphorous Reagent, Worthington Diagnostics), cholinesterase (Reagent Set Kit, Biodynamics/BMC Div), gamma-glutamyl transferase (Reagent Set Kit, Biodynamics/BMC Div), and creatine phosphokinase (CK-NAC Reagent Set Kit, Biodynamics/BMC Div.). Serum creatinine and serum uric acid were determined on automated equipment according to manufacturer's procedures (Technicon Auto Analyser, Technicon Corporation).

Haematology
Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) containing disodium ethylenediamine-tetraacetate. Blood smears for differential leukocyte counts were made immediately from blood containing no anticoagulant and stained with Wright's stain (Hema-Tek Slide Stainer, Ames Company). One hundred leukocytes were classified. Haemoglobin concentration was measured as cyanmethaemoglobin with a haemoglobinometer (Coulter Hemoglobinometer, Coulter Electronics). Erythrocyte count, mean corpuscular volume, haematocrit and total leukocyte count were determined with an electronic particle counter (Coulter Model ZBI with MCV/Hemat Computer and Channelyzer). Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were calculated.

Gross pathology and histopathology
All goats given glyphosate and one randomly selected goat from each control group were subjected to post-mortem examination at the end of the post-treatment observation period (days 14-15). All surviving goats to be necropsied were killed by the intravenous administration of a commercial euthanasia solution (T-61, National Laboratories Corp., Somerville, NJ 08876).
Specimens of heart, liver, kidney and spleen were collected from all necropsied animals for histopathological

examination. Additional specimens were collected when grossly visible lesions were observed. Slides for histologic examination were prepared from heart, liver, kidney, spleen and other tissues with grossly visible lesions from all animals given glyphosate at 4620 and 3090 mg/kg body weight, and from each of the four untreated control goats that were sacrificed. Tissues were fixed in neutral buffered 10 % formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin and examined microscopically.

Statistics
The LD_{50} was calculate by the moving average method. Group median, minimum and maximum values were calculated for each post-treatment sampling time for all clinical biochemical and haematological parameters. Control values (principals with concurrent vehicle controls) plus all post-treatment values from the concurrent vehicle control goats and calculating for this pool the following statistics: median, minimum value, maximum value, 5^{th} quantile, and 95^{th} quantile. Study-wide minimum and maximum control values were obtained by pooling control data from all 4 dose levels. Medians for post-treatment determinations that fell above or below the 95^{th} or 5^{th} quantiles respectively were considered significantly greater or less than the control median ($P < 0.05$). Single values for individual animals were considered to have changed significantly if they fell outside the absolute range of control values. The Mann-Whitney test was used for comparison of median percent change in body weight between goats that received glyphosate and their concurrent controls.

II. RESULTS AND DISCUSSION

A. MORTALITY, SURVIVAL TIME, AND LD_{50}

All animals treated with 10000 and 4620 mg/kg bw glyphosate died. 1/5 animals treated with 3090 mg/kg bw also died. The median lethal dose (LD_{50}) was calculated to be LD_{50}, goat, oral = 3530 mg/kg bw. The mortality, survival times and median LD_{50} are given in the table below.

Table B.6.8.2.8-1: The acute toxicity of glyphosate in female goats ([redacted], 1987): Mortality, survival time and median[1] LD_{50} in female goats

Group No. (N=5)	Glyphosate dose (mg/kg bw)	Mortality rate (%)	Survival time (hours)		
			Mean	Min.	Max.
13	10000	100	13.7	3.9	19.2
15	4650	100	16.5	2.4	32.2
16	3090	20	-	71.7	71.7
14	1980	0	-	-	-
LD_{50} = 3530 (2950-4220)[2] mg/kg bw					

[1] Glyphosate doses of 4820 and 7520 mg/kg bw were used in calculating the LD_{50} with the assumption that the mortality for both these levels would be the same as at 4620 mg/kg bw (100 %)
[2] 95 % confidence limits for LD_{50}

B. CLINICAL OBSERVATIONS

One control goat gave birth to a single live kid approximately one hour before the time of treatment. Another control goat gave birth to one normal and one small and very weak kid on day 4 of the experiment. One goat of the 4620 mg/kg bw-treated animals gave birth to two full-term kids during the night before dosing.
Abnormal clinical signs observed across all treatment groups are tabulated below.

Table B.6.8.2.8-2: The acute toxicity of glyphosate in female goats ([redacted], 1987): Clinical findings in female goats

Group No.	Dose (mg/kg bw)	Observations
13	10000	CFA (5)[1], apparent colic (2), depressed demeanour (2), slight ataxia (3), recumbency (4), death (5)
15	4620	CFA (5), apparent colic (2), depressed demeanour (2), ataxia (2), laboured breathing (2), recumbency (4), diarrhoea (1), death (5)

16	3090	Animal that died (1): CFA, diarrhoea, apparent colic, subdued demeanour, thirst, ataxia, recumbency, nystagmus, death All surviving animals: DFC (4), apparent colic (2) and diarrhea (4)
14	1980	DFC (5), diarrhoea (3)

[1] Number of goats affected; CFA = cessation of feeding activity; DFC = decreased feed consumption

C. BODY WEIGHT CHANGES

Immediately prior to death most animals exhibited a loss in body weight. Of the animals that survived until terminal sacrifice, those in the 3090 mg/kg bw group had mean body weights significantly less than their respective control group. Control animals and goats in the 1980 mg/kg bw group had similar mean body weights. The feed consumption was not measured, but cessation of feeding activity was observed in all treatment groups.

Table B.6.8.2.8-3: The acute toxicity of glyphosate in female goats ([redacted], 1987): Body weight changes in female goats

Glyphosate dose (mg/kg bw)	Post-treatment day weighted	Number of goats weighed	Percent change from initial weight		
			Median	Minimum	Maximum
Goats weighed immediately prior to death					
10000 - 4620	0	6[1]	-1.8	-3.9	3.8
4620	1	1	0	0	0
3090	3	1	-8.2	-8.2	-8.2
Surviving goats weighed at scheduled times					
3090	7	4	-12.7[3]	-18.1	-5.1
Control	7	4[2]	4.7	0	19.3
3090	14	4	-4.7	-14.7	7.0
Control	14	4[2]	-2.7	-6.6	14.8
1980	7	5	-6.9	-11.7	-3.7
Control	7	5	-2.8	-13.3	1.6
1980	14	5	-5.6	-6.2	-1.9
Control	14	5	-4.2	-7.2	0.8

[1] Carcass weights of 3 other goats that died on day 0 were not determined
[2] Data for one control goat omitted because of changes in body weight brought about by parturition
[3] Significantly different (P<0.01) from control median
Control = concurrent control goats for preceding group

D. POST-MORTEM GROSS PATHOLOGICAL OBSERVATIONS

No gross lesions that could be attributed to treatment were seen.
All results of the post-mortem gross pathology observations are tabulated below.

Table B.6.8.2.8-4: The acute toxicity of glyphosate in female goats ([redacted], 1987): Summary of gross pathological findings in treated and control goats

Dose (mg/kg bw)	D/S	Body as a whole	Respiratory	Cardio-vascular	Haemic and lymphatic	Gastro-intestinal	Urogenital
10000	5/0	NGPD	Pulmonary oedema (4/5)[1] Pneumonia (1/5)	NGPD	NGPD	NGPD	NGPD
4620	5/0	Serous atrophy of fat (1/5)	Pulmonary oedema (4/5)	NGPD	NGPD	Hepatic atrophy (1/5)	Macerated foetus (1/5)

3090	1/4	Serous atrophy of fat (1/5)	Pneumonia (1/5)	Pericarditis (1/5)	NGPD	Fatty liver (1/5) Rumen haemorrhage (1/5)	Pallor, kidneys (4/5) Renal hypertrophy (1/5) Endometritis (2/5) Foetal Death (1/5)
1980	0/5	NGPD	Pulmonary oedema (3/5) Pneumonia (1/5)	NGPD	NGPD	Fatty liver (1/5) Chronic hepatitis (1/5)	Renal atrophy (1/5)
Controls[2]	0/4	Minimal fat stores (1/4)	Pulmonary oedema (2/4)	NGPD	NGPD	NGPD	Pallor, kidneys (2/4) Cystitis (1/4) Metritis (1/4) Endometritis (1/4)

D/S = Died/Sacrificed; NGPD = No gross pathological diagnosis; (number of animlas with finding /number of animals)
[1] Animal affected/total animals in group
[2] only one randomly selected goat from each of the 4 control groups subjected to post mortem examination

E. HISTOPATHOLOGICAL OBSERVATIONS

Histological examination was performed only on heart, liver, kidney, spleen and other tissues with grossly visible lesions from animals given glyphosate at 4620 and 3090 mg/kg bw and of 4 sacrificed control animals. The most consistent finding in animals that died during the observation period was mild to severe tubular nephrosis. 3090 mg/kg bw-treated animals that survived lacked such lesions. 4/5 goats that died during the observation period had mild fatty change in the liver.

Table B.6.8.2.8-5: The acute toxicity of glyphosate in female goats ([redacted] 1987): Major histopathological diagnoses in selected female goats given glyphosate and control goats

Dose (mg/kg bw)	Group No.	Goat No.	Control for Group Number	Organ or Organ System				
				Heart	Spleen	Liver and Gallbladder	Urogenital	Other
4620	15	470[1]	-	NMD	NMD	Mild fatty change	Moderate to severe tubular nephrosis	N/A
		502[1]	-	Sarco-cystosis	NMD	Mild fatty change	Moderate to severe tubular nephrosis	N/A
		533[1]	-	NMD	NMD	Mild fatty change	Degeneration of placenta	N/A
		536[1]	-	NMD	NMD	Mild fatty change	Moderate to severe tubular nephrosis	N/A
		539[1]	-	NMD	NMD	NMD	Moderate to severe tubular nephrosis	N/A
0	28	528	15	NMD	NMD	Mild fatty change	NMD	N/A
3090	16	509[1]	-	Epi-carditis	NMD	Hepatic micro-abscesses	Severe tubular nephrosis, inflammation of placenta	Rumen necrosis and ulceration, pleuritis, broncho-pneumonia
		317	-	NMD	NMD	NMD	NMD	N/A

		424	-	NMD	NMD	Moderate fatty change	NMD	N/A
		543	-	NMD	NMD	NMD	NMD	N/A
		548	-	NMD	NMD	NMD	Endo-metritis	N/A
0	23	511	16	Multiple scars	NMD	NMD	Endometritis and retention of foetal membrane	N/A
0	19	494	13	NMD	NMD	NMD	NMD	N/A
0	21	461	14	NMD	NMD	NMD	NMD	N/A

[1] Fatally poisoned goats; all other goats shown were euthanized at the end of the study
NMD = No Morphologic Diagnosis.
N/A = Organ or system not examined histologically.

F. CLINICAL BIOCHEMISTRY AND HAEMATOLOGY

Elevation of blood urea nitrogen concentration, serum creatinine concentration, and numbers of circulating segmental neutrophils were the most consistent laboratory findings in goats given glyphosate. These changes were observed at all dose levels used in this study.
Clinical laboratory findings were almost universally within or near reference range for surviving glyphosate-treated goats at the end of the experiment (day 14).

Biochemical and haematological measurements, where one or more values fall outside of reference range, are tabulated below.

Table B.6.8.2.8-6: The acute toxicity of glyphosate in female goats ([redacted], 1987): Blood parameter findings in goats

Paramter (unit)	**Ref. value***	**Control[1]**	**Death in <4 h (1 goat)[2]**	**Death in 15-20 h (3 goats)[3]**	**Death in 32-72 h (2 goats)[4]**	**Death in 72 h (1 goat)[5]**	**Sign of intoxication observed (4 goats)[6]**		**No signs of intoxication observed (5 goats)[7]**	
BUN (mg/dL)	10.0-20.0	2.3-21.3	12.7	16.2-25.0^{++}	16.7-35.2^{++}	55.5^{++}	15.1-45.7^{++}	3.1-26.4^{++}	5.2-54.5^{++}	7.0-31.3^{++}
SC (mg/dL)	1.0-1.8	0.7-1.7	1.5	2.7-3.3^{++}	4.0-6.0^{++}	14.1^{++}	1.8-5.3^{++}	0.8-1.6	0.8-5.0^{++}	0.8-1.3
GLU (mg/Dl)	50.0-75.0	43-80	407^{++}	48-57	35-38	268^{++}	36-87	51.-87	55-100	59-98
Na (mg/dL)	327-356	311-354	303^{++}	333-335	313-321	327	317-331	301-332	317-347^{++}	319-350^{++}
K (mg/dL)	13.6-26.1	12.7-21.2	44.8^{++}	21.8-31.5	15.2-20.5	12.9	6.7-18.1	9.4-17.0	12.3-18.3	11.9-18.2
Ca (mg/dL)	8.9-11.7	8.0-15.7	18.9^{++}	15.2-18.6^{++}	8.7-10.3	6.3^{++}	6.2-10.3^{++}	7.9-10.2	6.1-10.1	8.5-11.4
P (mg/dL)	2.9-7.3	3.5-11.3	16.0^{++}	5.4-9.5	8.8-17.00^{++}	21.6^{++}	4.4-22.4^{++}	4.1-8.0	3.7-9.0	5.3-8.7
Mg (mg/dL)	2.8-3.6	1.30-3.20	4.01^{++}	2.87-3.61^{++}	1.98-3.07	2.38	1.53-2.48	1.53-2.38	1.98-3.22	1.29-2.38
SGOT (IU/L)	167-513	31-151	78	49-126	60-85	129	44-69	47-229^{++}	40-91	46-106
LDH (IU/L)	123-392	108-385	295	172-543^{++}	268-288	694^{++}	100-233	127-415^{++}	145-276	144-226
CPK (IU/L)	20-42	44-878	213	124-184	59-173	3285^{++}	83-187	73-245	84-264	81-158
WBC[5]	4.0-15.0	5.5-24.8	14.5	22.5-28.0^{++}	13.5-27.0^{++}	57.3^{++}	17.5-41.5^{++}	8.0-19.5	11.0-22.4	8.0-17.5
SEGS[5]	1.6-7.5	1.4-10.4	7.3	10.8-18.2^{++}	4.9-14.9^{++}	38.4^{++}	11.5-30.7^{++}	2.4-14.8^{++}	4.1-17.5^{++}	2.0-7.2

BANDS[5]	---	0-1.4	2.8^{++}	6.4-7.6^{++}	4.1-10.0^{++}	11.5^{++}	0.2-7.5^{++}	0.1-0.8	0-0.7	0-1.4
TSP (g/dL)	6.4-7.0	5.0-8.2	8.9^{++}	5.7-6.3	5.6-6.1	6.4	6.5-7.5	5.5-7.5	6.0-7.3	6.0-7.4

*Reference values as gathered from different published literature sources
[1] Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
[2] Goat sampled at death 3.8 h after receiving 10000 mg/kg bw
[3] Goats sampled 11 h after receiving 10000 mg/kg bw
[4] Goats sampled 24 h after receiving 4620 or 3090 mg/kg bw
[5] Goat sampled at death 72 h after receiving 3090 mg/kg bw
[6] Goats given 3090 mg/kg bw
[7] Goats given 1980 mg/kg bw
$^{++}$ One or more values outside of reference range

III. CONCLUSIONS

Assessment and conclusion by applicant:
Four groups consisting of five female (Spanish) goats obtained from farms were administered single oral gavage doses of glyphosate (via stomach tube) at doses of 1980, 3090, 4620, and 10000 mg/kg bw. Water was used as a vehicle in four negative control groups of five goats each. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology were assessed during a post-treatment observation period of 14 days.

At least three goats included in this study were discovered to be pregnant and, thus, females of different hormonal and physiological status were used. Weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous. Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

The acute oral LD_{50} was calculated to be 3530 mg/kg bw.

The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is suitable to provide additional information about acute oral toxicity in a ruminant species. The maximum dose exceeded those used in acute toxicity studies in laboratory rodents and more parameter were assessed in this study than would be included in an acute oral toxicity study in rodents. However, pathology was confined to four of a total of 20 control animals. In addition, sometimes data obtained in the four control groups were pooled and sometimes reported separately. The acute oral toxicity of glyphosate in goats was low.

Assessment and conclusion by RMS:

Due to the limitations noted the study is concluded to be supportive. Despite the limitations the study does indicate a fairly low acute oral toxicity in goats, similar as was observed in the rodent studies.

This conclusion is in line with the previous EU evaluation (2015).

B.6.8.2.9. In vivo acute oral toxicity in goat

Data point:	CA 5.8.2/009
Report author	[redacted]
Report year	1987
Report title	The acute oral toxicity of the isopropylamine salt of glyphosate (MON-

	0139) in female goats
Report No	80007[redacted]
Document No	[redacted]-80-451
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable, non-guideline study
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, The study is claimed to be in general conformance with GLP practices but no quality assurance was conducted and no GLP certificate for the lab is included in the study report. However, GLP was not yet compulsory at the time of conduct of the study.
Acceptability/Reliability:	Conclusion GRG: Supportive, Category 2a Conclusion AGG: Several limitations were noted. A very wide range of age of animals was used (8 months to 4 years) with a wide range in weight (14.7- 47.0 kg). Moreover, environmental conditions during housing was not reported. The dosing volume used seems fairly high (500 ml/goat). Abortion was noted in a few animals. It is unclear from the study report if other animals were pregnant. These limitation indicate that the study population was not homogenous. However, it is recognized that no guideline exist for this type of study. Overall, the study is concluded to be supportive.

Full summary

MON-0139, an aqueous formulation containing glyphosate isopropylamine salt (62.5%) was administered as a single dose via stomach tube to five groups (N=5) of female Spanish goats at doses of 10000, 6700, 5360, 4290, or 1400 mg/kg bw. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, and histopathology were observed for a post-treatment period of 14 days. Clinical signs, haematology, clinical biochemistry, and pathology were investigated in an effort to identify changes that are characteristic of MON-0139 intoxication and could be useful for diagnostic purposes.

The median acute oral LD_{50} was found to be 5700 mg/kg bw and the minimum lethal dosage (MLD) was 4290 mg/kg bw. The minimum dosage producing definite signs of illness (reduced feed consumption, diarrhoea) was 4290 mg/kg bw. Signs of toxicity in goats that died during the observation period included decreased feed consumption, abdominal distress, ataxia and, shortly prior to death, recumbency. One goat that died and one surviving goat each displayed an unusual "collapsing syndrome" of apparent neurological origin approximately 2 days after receiving MON-0139, while other goats displayed various other neurological signs. Sign of toxicity in goats that were sacrificed according to schedule included decreased feed consumption, diarrhoea, and loss of bodyweight. One goat developed extensive ulceration of the tongue and oral mucosa. These lesions healed completely by the end of the 14-day observation period.

No lesions considered to be treatment-related were noted at gross necropsy. No pathognomonic lesions were seen microscopically in treated goats. Mild to severe tubular nephrosis was the only consistent histopathological lesion observed in treatmentgoats died; however, this lesion was not observed in treatment goats that lived until the end of the experiment (days 14-15). This lesion is considered to be diagnostically significant for goats that die a few days after an appropriate level of exposure. Ischemic hippocampal neurons were observed in the brain of one goat that had displayed the so called "collapsing syndrome".

Changes in clinical biochemical parameters associated with toxicity included moderate elevation of serum urea nitrogen (BUN) and mild to moderate elevation of serum creatinine (SC). Slight elevations in serum glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) activity were also observed in terminal animals immediately prior to death. Temporary reductions in K and LDH were the only apparent treatment-related responses in goats dosed at 1400 mg/kg bw. No other diagnostically or toxicologically significant changes were observed. None of the biochemical parameters measured appeared to be involved in, or indicate the cause of, the unusual neurological manifestations seen in some goats receiving MON-0139.

Changes in circulating leukocytes associated with treatment with MON-0139 involved an increase in absolute numbers of mature (SEGS) and immature (BANDS) neutrophils with a decline in numbers of basophils and eosinophils. No consistent treatment-related changes in erythrocyte parameters were observed in this study.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:	MON-0139
Identification:	N-(phosphonomethyl)glycine isopropylamine salt
Description:	Amber liquid
Lot/Batch #:	LURT 08020
Purity:	62.5% (46.2 % for glyphosate)
Stability of test compound:	Stable for >1 year at 29.5 ºC. Store in a cool, dry area at 18.3-29.5 ºC
Vehicle:	Water was used to dilute the test article (1:1) and to wash any residue from the container holding the dose and from the stomach tube. The total volume of water was approximately 500 mL/goat.
Test animals:	
Species:	Goat
Strain:	Spanish[1]
Source:	[redacted]
Age:	8 months to 4 years
Number/Sex:	45 females
Weight at dosing:	14.7- 47.0 kg
Acclimation period:	At least 30 days
Diet/Feed:	During acclimatisation: bermuda grass hay and a commercial goat meal containing not less than 16.0% crude protein (Purina Goat Chow Coarse). After dosing: bermuda grass hay
Water:	Fresh water and mineral salt blocks available at all times
Housing:	During acclimatisation: Outdoor covered pens After dosing: Indoor pens
Environmental conditions:	Not reported

[1]The term Spanish is used to distinguish range meat goats from Angoras and dairy breeds. Most are of the same origin as the Mexican Criollo but they may show traces of Nubian and Toggenburg blood.

B. STUDY DESIGN AND METHODS

Animal assignment and treatment:
Groups of five of female Spanish goats received the test item, MON-0139, at dose levels of 10000, 6700, 5360, 4290, and 1400 mg/kg bw by oral gavage (rumen intubation) in a sequential manner. Initially, the test item was given at 10000 mg/kg/bw and subsequent doses were selected based on the observed responses. Four groups of negative control consisted of five animals each. They were concurrently treated with the vehicle (tap water). All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Blood samples were collected on three separate days during pre-treatment and 1, 3, 7, and 14 days after dosing (designated as day 0). Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.
During the initial holding period, all goats were vaccinated for enterotoxaemia, treated for internal parasites and

observed for signs of disease. Only goats without visible evidence of active disease and weighing more than 13 kg,

Randomization
All goats available for experimental use were ranked according to increasing bodyweight - The resulting ranking was divided into 5 nearly equal groups. Treatment and control groups of 5 goats each were formed by randomly selecting 1 goat from each of the 5 divisions of the ranking in order that the resulting groups would be representative with respect to bodyweight of the pool from which they were selected.

Body weight
Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14.

Clinical Biochemistry
An atomic absorption spectrophotometer (Perkin Elmer Model 403, Perkin-Elmer Corp.) was used to determine serum levels of calcium, magnesium, potassium, and sodium, as outlined in the manufacturer's manual. Serum levels of glucose, urea nitrogen, total protein (TP), alkaline phosphatase, lactic dehydrogenase, and glutamic oxaloacetic transaminase were determined on automated equipment (Gilford model 3500 computer directed analyser, Gilford Instruments, Inc.) according to manufacturer's procedures. Four other serum components were determined with the same automated equipment; however, different procedures and reagents were used for. these determinations, i.e., inorganic phosphorus (Inorganic Phosphorous Reagent, Worthington Diagnostics), cholinesterase (Reagent Set Kit, Biodynamics/BMC Div), gamma-glutamyl transferase (Reagent Set Kit, Biodynamics/BMC Div.) and creatine phosphokinase (CK-NAC Reagent Set Kit, Biodynamics/BMC Div.). Serum creatinine was determined on automated equipment according to manufacturer's procedures (Technicon Auto Analyser, Technicon Corporation).

Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) and allowed to clot at ambient temperature; the serum was separated by centrifugation. Serum LDH activity, CPK activity, and GLU concentration was determined as soon as possible after sample collection.

Haematology
Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) containing disodium ethylenediamine-tetraacetate. Blood smears for differential leukocyte counts were made immediately from blood containing no anticoagulant and stained with Wright's stain (Hema-Tek Slide Stainer, Ames Company). One hundred leukocytes were classified. Haemoglobin concentration was measured as cyanmethaemoglobin with a haemoglobinometer (Coulter Hemoglobinometer, Coulter Electronics). Erythrocyte count, mean corpuscular volume, haematocrit and total leukocyte count were determined with an electronic particle counter (Coulter Model ZBI with MCV/Hemat Computer and Channelyzer). Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were calculated.

Pathology
All goats given MON-0139 and one randomly selected goat from each control group were subjected to post-mortem examination at the end of the post-treatment observation period (days 14-15). All surviving goats to be necropsied were killed by the intravenous administration of a commercial euthanasia solution (T-61 Solution, National Laboratories Corp., USA).
Specimens of heart, liver, kidney, spleen and other tissue with grossly visible lesions were collected only from animals dosed at 6700 and 5360 mg/kg bw, and from each of the four untreated control goats that were sacrificed. Additional specimens were collected from one goat dosed at 4290 mg/kg bw and one goat dosed at 1400 mg/kg bw. Histopathologic examination on animals of the highest dose group were not performed. Tissue sections were stained with haematoxylin and eosin and examined microscopically.

Statistics
The LD_{50} was calculated by the method of Litchfield and Wilcoxon. Group median, minimum and maximum values were calculated for each post-treatment sampling time for all clinical biochemical and haematological parameters. Control values for each treatment groups were obtained by pooling all pre-treatment values (principals with concurrent untreated controls) plus all post-treatment values from the concurrent untreated control goats and calculating for this pool the following statistics: median, minimum value, maximum value, 5^{th} quantile, and 95^{th} quantile. Medians for post-treatment determinations that fell above or below the 95^{th} or 5^{th} quantiles respectively were considered significantly greater or less than the control median ($P<0.05$). Single values for individual animals were considered to have changed significantly if they fell outside the absolute

range of control values. Study-wide minimum and maximum control values were obtained by pooling control data from all 5 dose levels. The Mann-Whitney test was used for comparison of median percent change in bodyweight between goats that received MON-0139 and their concurrent controls.

II. RESULTS AND DISCUSSION

A. MORTALITY, SURVIVAL TIME, AND LD_{50}

Treatment with 10000, 6700, 5360, 4290, and 1400 mg/kg bw MON-0139 resulted in 5/5, 3/5, 2/5, 2/5, and 0/5 deaths, respectively. In the four control groups, there were no deaths. The acute oral LD_{50} for the dose-mortality data of this study was 5700 mg/kg bw MON-0139 with confidence limits of 3730 and 8710 mg/kg bw. The mortality and survival times are given in the table below.

Table B.6.8.2.9-1: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Mortality, survival time and median acute oral LD_{50} in female goats given MON-0139

Number of animals	MON-0139 Dosage[1] (mg/kg bw)	Mortality rate (%)	Survival time (hours)		
			Mean	Min.	Max.
5	10000	100	30.6	15.6	42.0
5	6700	60	52.5	37.0	64.0
5	5360	40	48.2	47.5	49.0
5	4290	40	69.2	53.2	85.1
5	1400	0	-	-	-
Lethal dose levels (mg/kg bw)					
LD_{50}= 5700 (confidence limits: 3730-8710) mg/kg bw					
LD_{16} = 2800 mg/kg bw					
LD_{84} = 11000 mg/kg bw					

[1] MON-0139 given as a single oral dose.

B. CLINICAL OBSERVATIONS

Clinical signs of goats that died included decreased feed consumption, abdominal distress, ataxia and, shortly prior to death, recumbency. One goat that died and one surviving goat dosed at 6700 mg/kg bw each displayed an unusual “collapsing syndrome” of apparent neurological origin approximately 2 days after receiving MON-0139 while other goats displayed various other neurological signs. One surviving goat developed extensive ulceration of the tongue and oral mucosa. These lesions healed completely by the end of the 14-day observation period.

Table B.6.8.2.9-2: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Prominent clinical observations in female goats given MON-0139

Number of animals	Dosage (mg/kg bw)	Observations
5	10000	DFC (5)[1], apparent colic (5); ataxia, shaking and jerking movements (2); depression (2); head bobbing (2); nystagmus (1); recumbency (4); diarrhoea (1); paddling (2); mild convulsions (2); death (5)
5	6700	Non-survivors (3): DFC (3); apparent colic (2); diarrhoea (1); bloating (3); ataxia (3); "saw-horse" stance (2); tremor (1); recumbency (3); nystagmus (2); jerky movements (2); blinking (1); terminal clonic-tonic activity (3); death (3) Survivors (2): DFC (2); bloating (1); abdominal distention (1); tremor (2); "collapsing syndrome" (1); diarrhoea (2)[2]

5	5360	Non-survivors (2): DFC (2); diarrhoea (1); ataxia (2); "saw-horse" stance (1); salivation (1); recumbency (1); tremor (l); "star-gazing" trance (l); death (2) Survivors (3): DFC (3); diarrhoea (3)
5	4290	Non-survivors (2): DFC (2); ataxia (1); "collapsing syndrome" (1); "chewing convulsions" (1); recumbency (2); salivation (1); terminal opisthotonus-like convulsions (l); death (2) Survivors (3): DFC (3); diarrhoea (3); apparent colic, salivation, lethargy, ulceration of oral mucosa (1)
5	1400	Minimal or no ill effects seen; reduced urination (?), abdominal distention (2), abortion (l)
20	0	Abortion (1)

DFC = decreased feed consumption
[1] Number of goats affected

C. BODY WEIGHT CHANGES

No statistically significant differences in body weight gain were observed between the groups treated with MON-0139 and their respective control groups. Feed consumption, although not precisely measured, was greatly reduced following treatment with MON-0139 at doses of 4290 mg/kg bw and above. For most groups, feeding activity gradually increased, reaching normal levels by the end of the study.

Table B.6.8.2.9-3: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Body weight changes in goats given MON-0139

Dose level (mg/kg bw)	Post treatment day weighed	Number of goats weighed	Percent change from initial weight		
Goats weighed immediately prior to death					
10000	0	1	NA	-2.1	-2.1
5360-10000	1	6	-0.5	-4.4	2.4
4290-9700	2	4	-7.2	-10.1	-3.8
4290	3	1	-NA	-8.2	-8.2
Surviving goats weighed at scheduled times					
6700	7	2	-7.0[1]	-10.4	-3.5
Control	7	4[2]	10.3	3.8	19.3
6700	14	2	-8.1	-4.4	-11.7
Control	14	4[2]	4.5	-6.6	-14.8
5360	7	3	-0.4	-9.0	3.2
Control	7	4[2]	0	-7.2	3.7
5360	14	3	0	-3.3	2.0
Control	14	4[2]	-1.8	-4.1	3.7
4290	7	3	-13.3	-17.4	-2.4
Control	7	5	-2.8	-13.5	1.6
4290	14	3	-8.1	-23.0	1.2
Control	14	5	-4.2	-7.2	0.8
1400	7	5	-0.7[1]	-3.4	10.5
Control	7	4[2]	10.3	3.8	19.3
1400	14	4[2]	-4.8	-6.2	7.6
Control	14	4[2]	4.5	-6.6	14.8

[1] Approaches being significantly different from control median ($0.05 < P < 0.10$)
[2] Data for one goat omitted because of changes in weight brought about by parturition
Control= Concurrent control goats for proceeding group
NA = not applicable, only 1 suriving animal

D. POST-MORTEM OBSERVATIONS
No lesions considered to be treatment-related were noted at gross necropsy.

Table B.6.8.2.9-4: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Significant gross pathologic diagnoses in female goats given MON-0139 and sacrificed untreated control goats

Dose level (mg/kg bw)	D/S[1]	Body as a whole	Respiratory	Cardio-vascular	Hemic and lymphatic	Gastro-intestinal	Urogenital
10000	5/0	NGPD[2]	pulmonary oedema (2/5)[3]	NGPD	NGPD	NGPD	NGPD
6700	3/2	NGPD	pulmonary oedema (1/5)	NGPD	NGPD	bloated (2/5)	pale kidneys (1/5) renal hypertrophy (1/5)
5360	2/3	minimal fat stores (1/5)	pulmonary oedema (3/5)	NGPD	NGPD	fatty liver (4/5)	pale kidneys (2/5)
4290	2/3	NGPD	pulmonary oedema (3/5)	NGPD	NGPD	fatty liver (1/5) gallbladder oedema (1/5)	pale kidneys (2/5) renal hypertrophy (1/5)
1400	0/5	minimal fat stores (1/5)	pneumonia (1/5) pulmonary oedema (2/5)	pericarditis (1/5)	NGPD	fatty liver (l/5)	metritis (1/5) pregnancy (1/5)
Controls	0/4	minimal fat stores (1/4)	pulmonary oedema (2/4)	NGPD	NGPD	NGPD	pale kidneys (2/4) mild cystitis (1/4) metritis (1/4) endometritis (1/4)

[1] Died/sacrificed
[2] No gross pathologic diagnosis
[3] Animals affected/total animals in group

E. HISTOPATHOLOGICAL OBSERVATIONS IN SELECTED MON-0139 TREATED AND UNTREATED GOAT
Histologic examination was performed only on heart, liver, kidney, spleen and other tissues with grossly visible lesions from animals given MON-0139 at 6700 and 5360 mg/kg bw, and from each of the four untreated control goats that were sacrificed. In addition, histologic examination was performed on tissues from one goat given MON-0139 at 4290 mg/kg and on tissues from one goat given MON-0139 at 1400 mg/kg. The most consistent finding in fatally poisoned animals was mild to severe tubular nephrosis.

Table B.6.8.2.9-5: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Major histopathologic diagnosis in selected female goats given MON-0139 and sacrificed untreated control goats

Dose level (mg/kg bw)	Goat Number	Organ or organ system				
		Heart	Spleen	Liver and Gallbladder	Urogenital	Other
1400	550	Scar	NMD	NMD	Severe bacterial metritis[1]	N
4290	472[2]	NMD	NMD	NMD	Mild tubular nephrosis	Ischemic hippocampal neurons with focal oedema
5360	522[2]	NMD	NMD	Mild to moderate	Moderate to severe	N

				fatty change	tubular nephrosis	
	512[2]	NMD	NMD	Moderate to severe fatty change	Mild to moderate tubular nephrosis	N
	520	NMD	NMD	Mild fatty change	NMD	N
	313	NMD	NMD	NMD	NMD	N
	549	Mild scaring		NMD	NMD	N
6700	407	NMD	NMD	NMD	NMD	N
	481[2]	NMD	NMD	Mild to moderate fatty change	Moderate to severe tubular nephrosis	N
	497	NMD	NMD	NMD	NMD	N
	316[2]	Focal myocarditis sarcocystosis	NMD	NMD	Moderate tubular nephrosis	N
	538[2]	NMD	NMD	NMD	Mild to moderate tubular nephrosis	N
0	528	NMD	NMD	Mild fatty change	NMD	N
	511	Multiple scars	NMD	NMD	Endometritis, retained foetal membranes[3]	N
	494	NMD	NMD	NMD	NMD	MMCB
	461	NMD	NMD	NMD	NMD	N

NMD = No Morphologic Diagnosis (no significant pathologic change observed)
N = Organ or system not examined histologically
MMCH = Mild multifocal chronic bronchiolitis

[1] Microscopic examination of foetal tissues revealed no specific lesions
[2] Fatally poisoned
[3] Foetal tissues not examined

F. CLINICAL BIOCHEMISTRY AND HAEMATOLOGY

Slight to moderate elevations in blood urea nitrogen (BUN) and serum creatine concentrations (SC) were observed in all animals that died during the study. These findings may be related to the histopathologic kidney lesions observed in these animals. Slight elevations in serum glutamic oxaloacetic transaminase (GOT) and lactic dehydrogenase (LDH) activity were also observed in terminal animals immediately prior to death.
No other diagnostically or toxicologically significant changes were observed. None of the biochemical parameters measured appeared to be involved in, or indicate the cause of, the unusual neurological manifestations seen in some goats receiving MON-0139. The results are summarized in the following table.

Table B.6.8.2.9-6: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Clinical biochemical and haematological measurements (min-max values) for goats given MON-0139

			Goats that died				**Goats that survived**			
			Surviving <49 h		**Surviving >50 h, <90 h**		**Definite signs of intoxication observed (8 goats)**		**Minimal or no signs of intoxication observed (5 goats)**	
Parameter (unit)	**Ref.**	**Control**	**Sampled 12-14 h before death (2 goats)**	**Sampled <4 h before death (5 goats)**	**Sampled at 24 h post-dosing (4 goats)**	**Sampled 6-16 h before death (3 goats)**	**Days 1 & 3**	**Days 7 & 14**	**Days 1 & 3**	**Days 7 & 14**
BUN (mg/dL)	10.0-20.0	2.3-21.3	26.7-44.1	31.5-62.4	13.8-22.0	44.2-111.4	7.6-28.7	3.7-135.6	3.9-13.6	6.0-19.1
SC	1.0-1.8	0.6-1.7	4.5-6.1	3.3-8.5	1.4-2.9	5.1-6.7	1.0-1.9	0.8-9.7	0.8-1.4	0.9-1.4

Table B.6.8.2.9-6: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Clinical biochemical and haematological measurements (min-max values) for goats given MON-0139

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Parameter (unit)	**Ref.**	**Control**	**Sampled 12-14 h before death (2 goats)**	**Sampled <4 h before death (5 goats)**	**Sampled at 24 h post-dosing (4 goats)**	**Sampled 6-16 h before death (3 goats)**	**Days 1 & 3**	**Days 7 & 14**	**Days 1 & 3**	**Days 7 & 14**
(mg/dL)										
GLU (mg/dL)	50.0-75.0	37-80	120-164	17-65	74-173	57-196	60-139	50-90	40-59	29-58
Na (mg/dL)	327-356	311-349	347-376	327-396	331-343	327-345	309-337	319-337	323-337	317-337
K (mg/dL)	13.6-26.1	13.4-21.2	16.2-16.5	10.9-23.2	10.3-16.9	9.0-11.0	12.2-17.6	8.0-19.5	13.9-19.5	15.0-19.4
Ca (mg/dL)	8.9-11.7	8.0-13.4	8.7-11.5	8.5-9.8	8.6-11.3	7.2-8.6	8.9-11.1	8.7-10.7	9.3-10.1	9.2-10.0
P (mg/dL)	2.9-7.3	4.1-11.9	2.9-16.0	3.5-10.0	3.9-6.4	2.9-5.9	3.5-10.2	3.8-9.7	4.5-9.3	4.9-8.8
Mg (mg/dL)	2.8-3.6	1.3-3.4	3.0-3.9	2.3-4.1	2.1-3.5	3.0-4.0	1.8-2.4	1.3-2.4	1.9-2.3	1.9-2.1
GOT (U/L)	167-513	31-151	99-100	128-459	58-107	105-145	34-77	3.7-169	53-81	50-83
LDH (U/L)	123-392	145-385	303-310	394-880	220-298	266-394	118-232	141-317	170-242	151-238
ACH (U/L)	-	240-496	405-461	388-536	344-459	409-471	293-528	209-450	273-386	255-407
ALKP (U/L)	93-387	31-902	69-283	76-282	97-422	173-476	58-594	52-542	31-323	30-307
CPK (U/L)	20-42	44-878	144-185	181-2631	110-149	183-1145	58-124	44-180	54-154	44-152
GGT (U/L)	-	12-60	19-26	23-38	30-35	30-38	23-43	21-42	15-40	11-30
WBC	4.0-15.0	5.5-17.5	12.0-14.0	3.7-21.5	9.0-23.0	10.0-18.0	5.7-19.5	8.0-35.5	7.5-12.5	7.5-20.0
SEGS	1.6-7.5	1.4-10.4	3.1-7.0	1.3-4.8	4.1-16.6	1.8-6.9	2.0-14.0	2.0-9.7	2.9-7.2	3.4-16.4
BANDS	-	0-1.4	3.6-5.9	1.3-12.1	0-4.8	1.4-9.7	0-1.0	0-1.8	0-0.3	0-0.6
LYMPH	1.8-9.0	2.4-9.2	2.6-2.8	1.0-6.5	1.4-4.9	1.7-4.4	2.0-6.7	3.1-8.4	2.9-6.4	2.0-6.3
EOS	0.1-1.5	0-1.4	0-0	0-0	0-0.1	0-0	0-0.1	0-0.7	0-0.7	0-0.4
MONO	0.1-0.9	0-0.9	0.4-0.6	0-0.9	0.1-0.4	0.1-0.5	0-0.8	0-0.7	0-0.2	0.1-3.9
BASO	0-0.2	0-0.8	0-0	0-0	0-0.1	0-0	0-0.3	0-0.6	0-0.5	0-0.4
PCV (%)	19-40	23-47	26-49	33-45	34-45	31-42	29-39	27-38	24-32	24-34
HGB (mg/dL)	8-16	7.8-16.7	9.6-15.6	10.6-15.6	10.8-14.4	10.6-14.8	10.4-12.9	9.4-12.4	11.3-12.2	9.8-11.3
RBC	7-21	12.8-24.2	15.6-23.8	16.0-26.9	19.0-24.5	16.7-22.9	15.6-23.2	15.7-21.9	18.0-19.0	15.7-17.9
MCV	15-39	15-21	17.0-18.0	15.0-19.0	15.0-18.0	15.0-18.0	15.0-9.0	13.0-9.0	14.0-21.0	15.0-1.0
MCH	5.3-8.4	4.6-7.8	6.2-6.6	4.8-7.3	5.5-6.5	5.4-6.5	5.1-7.5	5.3-7.0	5.0-6.9	5.2-7.2

Table B.6.8.2.9-6: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Clinical biochemical and haematological measurements (min-max values) for goats given MON-0139

			Goats that died				Goats that survived			
			Surviving <49 h		Surviving >50 h, <90 h		Definite signs of intoxication observed (8 goats)		Minimal or no signs of intoxication observed (5 goats)	
Parameter (unit)	**Ref.**	**Control**	**Sampled 12-14 h before death (2 goats)**	**Sampled <4 h before death (5 goats)**	**Sampled at 24 h post-dosing (4 goats)**	**Sampled 6-16 h before death (3 goats)**	**Days 1 & 3**	**Days 7 & 14**	**Days 1 & 3**	**Days 7 & 14**
MCHC (mg/dL)	32-40	28-41	31.8-36.9	31-35	32-37	32-35	32-38	31-37	33-40	34-41
TP (mg/dL)	6.4-7.0	5.0-8.2	6.7-8.6	5.8-7.3	7.2-7.7	6.2-7.7	5.9-8.7	5.6-7.7	6.1-7.2	5.9-7.1

Control values were obtained by pooling all pre-treatment values (principles and untreated controls) plus all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter from 60 goats).
BUN = Urea nitrogen, SC = Creatinine, GLU = Glucose, Na = Sodium, K = Potassium, Ca = Calcium, P = Phosphorus, Mg = Magnesium, GOT = Glutamic oxaloacetic transaminase, LDH = Lactic dehydrogenase, ACH = Cholinesterase, ALKP = Alkaline phosphatase, CPK = Creatinine phosphokinase, GGT = Gamma-glutamyltransferase, WBC = Total leukocyte count, SEGS = Segmented neutrophil count, BANDS = Band neutrophil count, LYMPH = Lymphocyte count, EOS = Eosinophil count, MONO = Monocyte count, BASO = Basophil count, PCV = Microhematocrit, HGB = Haemoglobin, RBC = Erythrocyte count, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular hemoglobin concentration, TP = Total protein

Study conclusion
The median acute oral LD_{50} was found to be 5700 mg/kg bw. The minimum lethal dose was 4290 mg/kg bw. The minimum dose producing definite signs of illness (reduced feed consumption, diarrhoea) was 4290 mg/kg bw. The lowest dose of MON-0139 given in this study (1400 mg/kg bw) produced minimal or no signs of toxicity. Signs of CNS dysfunction including behavioural abnormalities and convulsion were seen at MON-0139 doses of 4290 mg/kg bw and above.

One day of exposure to correctly treated forage should present no hazard to domestic ruminants with respect to any direct action of MON-0139 on animals consuming it. While MON-0139 did produce diarrhoea and apparent abdominal discomfort, vomiting and abnormally frequent regurgitation were not observed.

Assessment and conclusion by applicant:
Five groups consisting of five female (Spanish) goats obtained from farms were administered single oral gavage (via rumen intubation) doses of the isopropylamine salt of glyphosate (MON-0139) at doses of 1400, 4290, 5360, 6700, or 10000 mg/kg bw. Water was used as a vehicle in four negative control groups of five goats each. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology during a post-treatment observation period of 14 days.

At least three goats included to this study gave birth to kids and two aborted during this study and, thus, females of different hormonal and physiological status were used. Weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous. Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is good and it is suitable to provide additional information about acute oral toxicity in a ruminant species. The maximum dose exceeded those used in acute

toxicity studies in laboratory rodents and more parameters were assessed in this study than would be included in an acute oral toxicity study in rodents. However, pathology was confined to four of a total of 20 control animals. In addition, sometimes data obtained in the four control groups were pooled and sometimes reported separately. The acute oral LD_{50} was found to be 5700 mg/kg bw. The oral acute toxicity of the isopropylamine salt of glyphosate in goats was comparable to the one of glyphosate technical acid when corrected for purity (see also CA 5.8.2/008). The acute oral toxicity for both forms of glyphosate was low in goats.

Assessment and conclusion by RMS:

Due to the limitations noted the study is concluded to be supportive. Despite the limitations the study does indicate a fairly low acute oral toxicity in goats, similar as was observed in the rodent studies.

This conclusion is in line with the previous EU evaluation.

B.6.8.2.10. In vivo subacute oral toxicity in cattle

Data point:	CA 5.8.2/010
Report author	[redacted]
Report year	1987
Report title	The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle
Report No	80002
Document No	[redacted]-82-003
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable, non-guideline study
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, The study is claimed to be in general conformance with GLP practices but no quality assurance was conducted and no GLP certificate for the lab is included in the study report. However, GLP was not yet compulsory at the time of conduct of the study.
Acceptability/Reliability:	Conclusion GRG: Supportive, Category 2a Conclusion AGG: Several limitations were noted. Environmental conditions were note reported. More importantly a low number of animals was used (n = 2 to 3). Although there is no guideline for this type of study the number of animals seems too low to derive any meaningful results from the study. Therefore, the study is concluded to be unacceptable.

Full summary

MON-0139, an aqueous formulation containing glyphosate isopropylamine salt (62.4%) was administered in seven consecutive daily doses by stomach tube to Brahman cross heifers. In a preliminary trial, MON-0139 was administered at a dose of 1000 mg/kg bw/day to two heifers. Based upon the observed response at that dose, subsequent doses of 540, 830, 1290 and 2000 mg/kg bw/day were administered to groups of three heifers each. Concurrent negative control animals (n=2 per treatment group) were treated with the vehicle only. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, and histopathology were observed for a post-treatment period of 14 days.

All three top dose heifers died within 1.5 days after receiving the sixth or seventh dose. In these animals, nervous system effects like head tremors, convulsions, ataxia and possible visual impairment and shortly prior to death, sternal recumbency occurred. In the group receiving 1290 mg/kg bw/day, one heifer also died after the final dose but without exhibiting signs of nervous dysfunction. Clinical signs of general toxicity included a decrease in feed

intake, weight loss, diarrhoea and behavioural depression, and were noted at the two upper dose levels. Diarrhoea and decreased feed consumption were also seen in the mid dose group receiving 830 mg/kg bw/day.

The minimum lethal dose (MLD), minimum toxic dose (MTD) and no observable effect level (NOEL) were estimated to be 1290, 830, and 540 mg MON-0139/kg bw/day, respectively.

Gross pathology revealed signs of dehydration and of gastrointestinal irritation at the upper dosages (mucosal congestion of the rumen, abomasum and duodenum and erosions of the mucosa of the abomasum). High kidney weight to brain weight ratios were seen in all heifers given MON-0139 at 2000 mg/kg bw/day and two of three heifers given MON-0139 at 1290 mg/kg bw/day. High liver weight to brain weight ratios were observed in all heifers given MON-0139 at 2000 mg/kg bw/day.

Histopathologic examination was performed only on liver, kidney, and other organs with gross lesions from heifers given MON-0139 at 1290 and 830 mg/kg bw/day. Histopathological findings were confined to the kidneys and consisted of mild to marked tubular vacuolization (primarily proximal convoluted tubules) and nuclear pyknosis in many tubular epithelial cells in the heifers receiving 1290 mg/kg bw/day. No lesion was seen microscopically in liver from these heifers. Renal tubular vacuolization was not observed in heifers given MON-0139 at 830 mg/kg bw/day.

Clinical laboratory findings associated with MON-0139 doses of 1290 and 2000 mg/kg bw/day consisted of haemoconcentration (elevated PCV, HGB, RBC, and STP); increased serum levels of urea nitrogen, creatinine, GOT, LDH, CK and reduced number of circulating lymphocytes and eosinophils. In addition, heifers that died during the study period exhibited increased numbers of circulating immature (BANDSA) and mature (SEGSA) neutrophils. The surviving heifers (1290 or 830 mg MON-0139/kg bw/day) exhibited mild reductions in serum levels of sodium, inorganic phosphorus, and serum cholinesterase activity. Significant elevation of median serum magnesium concentration was observed at MON-0139 doses above 540 mg/kg bw/day. Significant reduction of median serum potassium (K) concentration was seen at all levels of MON-0139 exposure; however, the decline in K for heifers given MON-0139 at the lowest dose level (540 mg/kg bw/day) was small in magnitude and values for K remained within the limits of study-wide control values for all individuals of this group.

I. MATERIALS AND METHODS

A. MATERIALS

Non-labelled test material:	MON-0139
Identification:	N-(phosphonomethyl)glycine isopropylamine salt
Physical state:	Aqueous solution
Description:	Amber liquid
Lot/Batch #:	LBRT 08023
Source:	Monsanto Company, St. Louis, MO
Purity:	62.4% (46.2% for glyphosate N-(phosphonomethyl)glycine)
Stability of test compound:	Stable for >1 year at 29.5 ºC. Store in a cool, dry area at 18.3-29.5 ºC
Vehicle:	Water (approximately 500 mL/heifer)
Test animals:	
Species:	Cattle
Strain:	Brahman cross
Source:	[redacted]
Age:	Not reported (heifers)
Number/Sex:	20 females
Weight at dosing:	169 to 248 kg (mean 215.6 ± 23.1 kg)
Acclimation period:	At least 30 days

Diet/Feed:	During acclimatisation: bermudagrass hay and a commercial feed concentrate containing not less than 13.0% crude protein (Purina Commercial Creep Chow) After acclimatisation: bermudagrass hay
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Outdoor pens for a period of 15 to 19 days then indoor pens for a period of 11 to 15 days After dosing: Individual indoor pens.
Environmental conditions:	Not reported
Concomitant treatment:	During the initial holding period, all heifers were vaccinated for Clostridial and Leptospiral diseases and treated for internal parasites

B. STUDY DESIGN AND METHODS

Study dates: 02-Feb-1982 to 05-May-1982

Randomization
All heifers available for experimental use were ranked according to increasing bodyweight. The resulting ranking was divided into 3 nearly equal groups. Treatment groups of three heifers each and control groups of two heifers each were formed by randomly selecting one heifer from each of the three divisions of the ranking in order that the resulting groups would have body weights representative of the pool from which they were selected.

Animal assignment and treatment
Brahman-cross heifers were treated in a sequential manner with MON-0139 by rumen intubation as seven consecutive daily doses of 540, 830, 1000 (preliminary trial, n = 2), 1290, and 2000 mg/kg bw/day (n = 3/group). In the preliminary trial two heifers treated at 1000 mg/kg bw/day over seven consecutive days, body weighing and visual assessment of clinical condition were the only assessments performed.
The test article was given as grams of total formulation per kg body weight. Water was used to dilute the test article (1:1) and to wash any residue from the container holding the dose and from the stomach tube. The total volume of water used for these procedures was approximately 500 mL/heifer. Initially, the test item was given at 2000 mg/kg/bw/day and subsequent doses were selected based on the observed responses. Negative control animals were sham treated with tap water. All animals were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment until end of study (day 21). Hay consumption was evaluated subjectively by observing feeding activity and visual inspection of the quantity of hay uneaten each day.

Body weight
Body weights were determined prior to dosing (day 0) and on days 6, 14, and 21. Carcass weights were determined for heifers that died during the study period.

Collection of Blood Samples
Samples of blood for clinical biochemical and haematological examination were collected on three separate days during the pre-treatment period. After the first dose of MON-0139 (day 0) samples were collected on days 2, 6, 8, 14 and 21. Samples were taken at unscheduled times when dictated by clinical signs or impending death.

Clinical Biochemistry
An atomic absorption spectrophotometer (Perkin Elmer Model 403, Perkin-Elmer Corp.) was used to determine serum levels of calcium, magnesium, potassium, and sodium, as outlined in the manufacturer's manual. Serum levels of glucose (GLU), urea nitrogen, total protein (TP), alkaline phosphatase, lactic dehydrogenase (LDH), and glutamic oxaloacetic transaminase were determined on automated equipment (Gilford model 3500 computer directed analyser, Gilford Instruments, Inc.) according to manufacturer's procedures. Four other serum components were determined with the same automated equipment; however, different procedures and reagents were used for these determinations, i.e., inorganic phosphorus (Inorganic Phosphorous Reagent, Worthington Diagnostics), cholinesterase and gamma-glutamyl transferase (Reagent Set Kit, Biodynamics/BMC Div.), and creatine phosphokinase (CK-NAC Reagent Set Kit, Biodynamics/BMC Div). Serum creatinine was determined

on automated equipment according to manufacturer's procedures (Technicon Auto Analyser, Technicon Corporation).

Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) and allowed to clot at ambient temperature; the serum was separated by centrifugation. Serum LDH activity, CPK activity, and GLU concentration was determined as soon as possible after sample collection.

Haematology
Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) containing disodium ethylenediamine-tetraacetate. Blood smears for differential leukocyte counts were made immediately from blood containing no anticoagulant and stained with Wright's stain (Hema-Tek Slide Stainer, Ames Company). One hundred leukocytes were classified. Haemoglobin concentration was measured as cyanmethemoglobin with a hemoglobinometer (Coulter Hemoglobinometer, Coulter Electronics). Erythrocyte count, mean corpuscular volume, haematocrit and total leukocyte count were determined with an electronic particle counter (Coulter Model ZBI with MCV/Hemat Computer and Channelyzer). Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were calculated.

Pathology
Except for the two heifers of the preliminary trial, all animals given MON-0139 and both heifers from each control group were subjected to post-mortem examination at the end of the post-treatment observation period. Surviving MON-0139 treated and control heifers to be necropsied were killed by the intravenous administration of a commercial euthanasia solution (T-61 Solution, National Laboratories Corp., USA). Organ weight was measured for kidney, liver, and brain. Specimens of heart, liver, kidney, spleen, and other tissues with gross lesions were collected and fixed in neutral, buffered 10 % formalin; however, slides for histologic study were prepared and examined only for liver, kidney and other tissues with gross lesions from animals given MON-0139 at 1290 and 830 mg/kg bw/day, and from six of the eight untreated control heifers. Tissues to be examined microscopically were embedded in paraffin, sectioned, and stained with haematoxylin and eosin.

Statistics
The MTD (minimum toxic dosage) and MLD (minimum lethal dosage) were estimated directly from the morbidity-mortality data. Group median, minimum and maximum values were calculated for each post-treatment sampling time for all clinical biochemical and haematological parameters. Control values for each treatment group were obtained by pooling all pre-treatment values (principals with concurrent untreated controls) plus all post-treatment values from the concurrent untreated control heifers and calculating for this pool the following statistics: median, minimum value, maximum value, 5^{th} quantile, and 95^{th} quantile. Medians for post-treatment determinations that fell above or below the 95^{th} or 5^{th} quantiles respectively were considered significantly greater or less than the control median ($p \leq 0.05$). Study-wide minimum and maximum control values were obtained by pooling control data from all four dose levels. Single values for individual animals were considered to have changed significantly if they fell outside the absolute range of control values. The Mann-Whitney test was used for comparison of mean percentage change in bodyweight, mean kidney to brain weight ratios (KBR) and mean liver to brain weight ratios (LBR) between heifers that received MON-0139 and their concurrent controls.

II. RESULTS AND DISCUSSION

A. MORTALITY, SURVIVAL TIME AND MTD

All 3 animals treated with 2000 mg/kg bw/day MON-0139 and 1/3 treated with 1290 mg/kg bw/day died.

Table B.6.8.2.10-1: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Survival times, morbidity and mortality

Dose (mg/kg bw/day)	Animal number	Number of doses given	Toxic signs	Death	Survival time[1] (days)	Morbidity (%)	Mortality (%)
2000	41	6	Yes	Yes	6.2	100	100
	45	7			6.5		
	31	7			7.5		
1290	44	7	Yes	Yes	7.5	100	33.3

	40 35			No	-		
1000 (preliminary trial)	13 17	7	Yes	No	-	100	0
830	37 38 39	7	Yes	No	-	100	0
540	32 33 42	7	No	No	-	0	0

No mortality occurred in the eight control animals

B. CLINICAL OBSERVATIONS

In the preliminary study, treatment with 1000 mg/kg bw/day MON-0139 decreased feed intake and induced diarrhoea by the second day of treatment which continued throughout the seven day treatment period. The signs had ceased by the end of the study on day 14.

In the main study, treatment with 2000, 1290, and 830 mg/kg bw/day induced diarrhoea. Animals exposed to 2000 mg/kg bw/day MON-0139 additionally showed nervous system effects including head tremors, convulsions, ataxia, and possible visual impairment and sternal recumbency.

All eight negative control heifers in this study appeared healthy throughout the pre-treatment, treatment and post-treatment periods; no sign of illness was observed.

Table B.6.8.2.10-2: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Prominent clinical observations in heifers given seven daily doses of MON-0139

Number of animals	Dose (mg/kg bw/day)	Observations
3	2000	diarrhoea (3)[1], decreased feed intake (3), nasal discharge (3), foamy salivation (1), head tremors (3), belligerency (1), whole-body tremors (3), ataxia (3), head pressing (1), kicking at imaginary objects (1), apparent visual impairment (1), convulsions (1), falling (1), depression (1), recumbency (3), increased respiratory effort (2), death (3)
3	1290	diarrhoea (3) , decreased feed intake (3), depression (3), weakness (2), death (1)
2 Preliminary trial	1000	diarrhoea (2), decreased feed intake (2)
3	830	diarrhoea (3), decreased feed intake (3)
3	540	no signs of toxicity
8	0	no signs of toxicity

[1] Number of animals affected

C. BODY WEIGHT CHANGES AND FEED CONSUMPTION

Treated animals showed decreased body weight and feed intake (the feed intake was not accurately measured). In the preliminary study, treatment with 1000 mg/kg bw/day MON-0139 decreased feed intake. Treatment with 2000 and 1290 mg/kg bw/day decreased feed intake. Treatment with 1290 mg/kg bw/day induced severe weight loss and depression for the first two weeks. While feed consumption and faecal consistency was similar to that of the controls during the third week after treatment, the animals remained thin and weakened. For the control animals, feed intake and character of the faeces remained normal throughout the study.

Table B.6.8.2.10-3: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Mean percent body weight changes in heifers given MON-0139

Dose (mg/kg bw/day)	Day of treatment	Mean Percentage change from initial body weight (N)			
		MON-0139 treated heifers		Control heifers	
2000	6	-1.2	(3)	-1.29	(2)
1290	6	-6.5[1]	(3)	-2.1	(2)
	14	-12.0[2]	(2)	-1.6	(2)
	21	-9.6	(2)	-3.4	(2)
830	6	-5.2[1]	(3)	-1.5	(2)
	14	-4.6	(3)	-0.4	(2)
	21	-2.4[1]	(3)	5.1	(2)
540	6	0.3	(3)	-0.7	(2)
	14	1.0	(3)	2.7	(2)
	21	0.4	(3)	1.5	(2)

[1] Approaches being significantly different from concurrent control mean ($p<0.083$)
[2] Approaches being significantly different from concurrent control mean ($p<0.121$)
N = number of heifers weighed

D. POST-MORTEM OBSERVATIONS

Few significant gross lesions were noted in heifers that died during the study period; however, at the time of necropsy each was judged to be dehydrated or to have suffered loss of body weight. Nonspecific (agonal) lesions included aspiration of rumen contents, petechial haemorrhage associated with the epicardium and spleen and pulmonary oedema. Post-mortem evidence of diarrhoea was observed in three of these heifers and no faeces were present in the rectum of a fourth heifer. Other lesions observed in these animals potentially attributable to treatment included erosions of the abomasal mucosa and congestion of the mucosa of the rumen, abomasum, and duodenum. One heifer administered 2000 mg/kg bw/day exhibited hyphaema (blood in the anterior chamber) in both eyes. No treatment-related lesions were observed at necropsy in the other eight MON-0139 treated heifers that were sacrificed at the end of the observation period (day 21 or 22).

High kidney weight to brain weight ratios were seen in all heifers given MON-0139 at 2000 mg/kg bw/day and two of three heifers given MON-0139 at 1290 mg/kg bw/day. High liver weight to brain weight ratios were observed in all heifers given MON-0139 at 2000 mg/kg bw/day. These observations became rare or absent at lower MON-0139 dose. Mean KBR was greater than but not significantly different from mean KBR of concurrent controls for MON-0139 doses of 2000 and 1290 mg/kg bw/day. Mean KBR was less than but not significantly different from concurrent controls for heifers given MON-0139 at 830 or 540 mg/kg bw/day. Findings for LBR were similar.

A mycotic dermititis was observed in many animals of this study, including untreated control heifers. Major gross pathologic findings noted in treated and control heifers are presented in the following table and unless indicated otherwise were judged to be unrelated to treatment and due to common agents such as parasites or the method of killing.

Table B.6.8.2.10-4: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Mean kidney and liver weight divided by brain weight for heifers given MON-0139 and concurrent controls

Dose (mg/kg bw/day)	Number of heifers	Kidney/Brain	Liver/Brain
2000	3	1.86[2]	7.57[2]
0[1]	2	1.25	6.45
1290	3	1.49[2]	5.96
0[1]	2	0.96	4.81
830	3	1.14[2]	5.75
0[1]	2	1.27	6.08
540	3	1.18	6.14
0[1]	2	1.19	5.92

[1] Concurrent controls for preceding MON-0139 treated group
[2] Approaches being significantly different from concurrent control mean (P <0.083)

E. HISTOPATHOLOGICAL OBSERVATIONS

Microscopic examination of kidney from the heifer given MON-0139 at 1290 mg/kg bw/day that died revealed marked renal tubular vacuolization. The proximal portion of the convoluted tubules appeared to be most severely involved. In addition, many tubular epithelial cells contained pyknotic nuclei. Histologic examination of the abomasum revealed multifocal superficial mucosal erosions, which appeared to be of recent development based on the minimal extent of the associated cellular reaction. Segmental congestion of the ileum (noted grossly) was characterized microscopically by focal necrosis and inflammation of the mucosa overlying Peyer's patches that extended superficially into the lymphoid tissue. Bacterial colonization of these areas in the ileum was marked. Blood in the anterior chamber of both eyes of one heifer was confirmed microscopically and was judged to have been the result of trauma.

The two surviving heifers given MON-0139 at 1290 mg/kg bw/day each had mild renal tubular vacuolisation. Focal vasculitis and perivasculitis was noted in the livers of one animal and one control animal and was considered to be the result of parasite migrations.

There were no significant treatment-related microscopic lesions observed in the tissues studied from heifers given MON-0139 at 830 mg/kg bw/day or tissues studied from control heifers. Dermatomycosis consistent with lesions caused by *Trichophyton spp.* was observed in both treated and control animals.

Table B.6.8.2.10-5: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Major gross pathologic findings in heifers given MON-0139 and in untreated control heifers

Animal number	Body as a whole	Skin	Respiratory	Cardio-vascular	Haemic & Lymphatic	Digestive	Urogenital
2000 mg MON-0139/kg bw/day							
31-12[1]	Dehydration; weight loss	Dermatitis	Aspiration (agonal)	Petechiae, epicardial	Petechiae, capsule of spleen	Erosions, abomasal mucosa[2]; congestion abomasum rumen; diarrhoea[2]; HLBR	NGL; HKBR[2]
45-12[1]	Dehydration (moderate)	Dermatitis	Nodules subpleural; Oedema pulmonary (mild)	Petechiae, epicardial	NGL	Congestion, abomasal and duodenal mucosa[2]; Distension, gall bladder, diarrhoea, HLBR[2]	NGL; HKBR[2]
41-12[1]	Weight loss	Dermatitis	Oedema pulmonary	Petechiae, epicardial	NGL	Congestion, ileal mucosa; diarrhoea[2]; congestion, liver; HLBR[2]	NGL; HKBR[2]
30-13[3]	NGL	Dermatitis	NGL	NGL	NGL	NGL	NGL
4-13[3]	Weight loss	Dermatitis	NGL	NGL	NGL	NGL	Infarct, rt. kidney
1290 mg MON-0139/kg bw/day							
40-22	Weight loss; serious atrophy of fat	Dermatitis	Petechiae, pleura	Petechiae, epicardial	NGL	Scars, capsule of liver	NGL
35-22	NGL	Dermatitis	NGL	NGL	NGL	NGL	NGL; HKBR[2]

Table B.6.8.2.10-5: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Major gross pathologic findings in heifers given MON-0139 and in untreated control heifers

Animal number	Body as a whole	Skin	Respiratory	Cardio-vascular	Haemic & Lymphatic	Digestive	Urogenital
44-22[1]	Dehydration	NGL	NGL	Hemocyst, heart valve	NGL	Erosions, abomasal mucosa; Congestion mucosa of ileum	NGL; HKBR[2]
34-23[3]	NGL	Dermatitis	Petechiae trachea mucosa and pleura	NGL	NGL	Petechiae, duodenum; congestion; Peyer's patches	Petechiae urinary bladder
9-23[3]	NGL	Dermatitis	NGL	NGL	NGL	Erosion oesophagus	NGL
830 mg MON-0139/kg bw/day							
38-32	NGL	NGL	NGL	NGL	NGL	Congestion colon, round ligament	NGL
37-32	NGL	NGL	NGL	NGL	NGL	Scars (2), liver capsule	NGL
39-32	NGL	Dermatitis	NGL	NGL	NGL	NGL	NGL
28-33[3]	NGL	NGL	NGL	NGL	Congestion, spleen	Petechiae, abomasal mucosa; Congestion ileal mucosa	Congestion vulvar mucosa
11-33[3]	NGL	Dermatitis	NGL	NGL	NGL	Congestion, ileum and cecum	NGL
540 mg MON-0139/kg bw/day							
42-42	NGL	NGL	Focus, subpleural	NGL	NGL	Erosions, oesophagus, HLBR	HKBR
32-42	NGL	NGL	Red foci, subpleural	NGL	NGL	Congestion, ileum and Peyer's patches; mottling, liver	NGL
33-42	NGL	NGL	NGL	NGL	NGL	NGL	NGL
29-43[3]	NGL	NGL	NGL	NGL	NGL	Lipoma	NGL
20-43[3]	NGL	Dermatitis	NGL	NGL	Lymphoid hyperplasia pharyngeal nodes (mild)	NGL; parasitism	NGL

[1] Fatally poisoned, all other animals -were sacrificed at the end of the observation period
[2] Observations (non-agonal) potentially attributable to treatment
[3] Untreated control heifers for preceding group
NGL = No Gross Lesions
HLBR = High liver weight to brain weight ratio relative to controls
HKBR = High kidney weight to brain weight ratio relative to controls

F. CLINICAL BIOCHEMISTRY AND HAEMATOLOGY

Treatment with 1290 and 2000 mg/kg bw/day MON-0139 increased haematocrit, haemoglobin, red blood cells and increased serum levels of total protein, urea nitrogen (BUN), creatinine and serum glutamic oxaloacetic transaminase (GOT), lactic dehydrogenase (LDH), and creatine phosphokinase (CPK) activities.

The haematologic alterations were considered to be due to haemoconcentration secondary to fluid shifts resulting from diarrhoea.
Elevations in CPK, GOT, and LDH activities were attributed to muscle damage resulting from convulsions and/or prolonged sternal recumbency.
Slight elevations of BUN and creatinine may have been due to decreased renal perfusion produced by dehydration secondary to diarrhoea. However, the presence of histopathological kidney lesions at 1290 mg/kg bw/day and changes in serum electrolyte levels at several doses suggest that these changes may have been partly due to some renal impairment.
An increase in the number of neutrophils and a decrease in the number of lymphocytes observed at 1290 and 2000 mg/kg bw/day probably represented a response to stress-induced release of corticosteroids.

Table B.6.8.2.10-6: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Days after treatment on which median values for clinical laboratory measurements performed on heifers given MON-0139 for 7 days were significantly different from concurrent median control values

	Dose (mg/kg bw/day)			
Parameter	**2000**	**1290[1]**	**830**	**540**
BUN	6	2, 6, 7, 8, 14	-	8[2]
CREAT	6	7, 8	-	8[2]
GLU	-	7	-	-
Na	6	6[2], 14[2]	-	-
K	6[2]	6[2], 8[2], 14[2]	6[2]	14[2]
Ca	6[2]	-	8[2]	-
P	6	7, 21[2]	2	-
Mg	6	7, 8	2	-
GOT	6[3]	2, 6, 7, 8, 14, 21	-	-
LDH	6[3]	6, 7	-	-
SACH	-	14[2]	21[2]	-
ALKP	-	7	-	-
CKN	6	2, 6, 7, 8	-	-
GGT	-	-	-	8, 14, 21
WBC	-	-	-	-
SEGSA	6	-	-	-
BANDSA	6	-	-	-
LYMPHSA	6[2]	6[2]	-	-
EOSA	2[4], 6[4]	6[4], 8[4]	-	14[2], 21[2]
MONOSA	-	-	-	-
BASOA	-	-	-	-
PCV	6	6[3], 7, 8	-	-
HGB	6	6, 7, 8	-	-
RBC	6[3]	6, 7, 8	-	-
MCV	-	-	-	-
MCH	-	-	-	-
MCHC	-	6, 21	-	-
STP	6	6, 7, 8	-	-

[1] Represents data from three animals on days 2 and 6 and two animals on days 8, 14, and 21. Entries for day 7 represent one animal, and the value is outside the range of study-wide control values
[2] Medians significantly lower than the concurrent median control value; entries without a symbol represent medians significantly greater than the concurrent median control values.
[3] Noteworthy non-significant increase
[4] Noteworthy non-significant decrease
BUN = Urea nitrogen, CREAT = Creatinine, GLU = Glucose, Na = Sodium, K = Potassium, Ca = Calcium, P = Inorganic phosphorus, Mg = Magnesium, GOT = Glutamic oxaloacetic transaminase, LDH = Lactic dehydrogenase, SACH = Cholinesterase, ALKP = Alkaline phosphatase, CKN = Creatine phosphokinase, GGT = Gamma-glutamyltransferase, WBC = Total leukocyte count, SEGSA = Segmented neutrophil count, BANDSA = Band neutrophil count, LYMPHSA =

Lymphocyte count, EOSA = Eosinophil count, MONOSA = Monocyte count, BASOA = Basophil count, PCV = Microhematocrit, HGB = Haemoglobin concentration, RBC = Erythrocyte count, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular hemoglobin concentration, STP = Total protein

Table B.6.8.2.10-7: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Clinical biochemical and haematological measurements (min-max values) for heifers given MON-0139

Parameter (unit)	Ref.[1,2]	Control[3]	Death on day 6 or 7		Surviving heifers			
			Sampled 6-14 h before death (3 heifers)	Sampled 34-38 h before death (2 heifers)	Signs of intoxication observed (5 heifers)		No signs of intoxication observed (3 heifers)	
					Days 2, 6, 8	Days 14 & 21	Days 2, 6, 8	Days 14 & 21
BUN (mg/dL)	6.0-27.0	4.3-24.0	55.5-100.0	26.5-65.6	7.3-85.2	6.5-47.3	5.0-13.8	8.2-21.5
CREAT (mg/dL)	0.20-2.60	1.30-2.55	8.62-11.30	4.7-11.1	1.55-9.55	1.50-2.55	1.55-2.35	1.40-2.20
GLU (mg/dL)	45-130	46-180	104-229	137-257	68-151	65-110	65-89	71-87
Na (mg/dL)	304-350	317-358	348-392	319-339	309-339	312-345	329-337	317-331
K (mg/dL)	15.2-26.5	11.8-21.0	11.7-19.1	11.6-12.4	8.9-15.4	7.1-17.8	15.4-17.0	14.2-15.8
Ca (mg/dL)	9.4-12.2	8.6-13.4	8.2-11.6	8.5-9.3	9.2-10.9	9.5-11.4	9.9-12.0	9.9-11.7
P (mg/mL)	4.5-9.3	5.4-9.9	6.9-20.7	9.9-111.3	5.7-10.5	3.9-9.0	6.0-7.6	6.9-8.0
Mg (mg/mL)	1.20-3.50	1.58-2.48	3.27-4.46	2.23-3.51	1.88-3.47	2.03-2.48	1.83-2.13	2.03-2.28
GOT (U/L)	8.5-93	27-70	56-132	62-133	33-189	41-87	37-72	32-64
LDH (U/L)	8-302	475-737	694-965	666-759	398-1186	465-705	540-731	494-729
SACH (U/L)	-	197-412	315-421	288-334	200-326	220-333	230-314	211-323
ALKP (U/L)	94-170	28-169	62-251	73-126	27-149	28-126	53-137	58-130
CKN (U/L)	-	45-714	274-6988	502-2079	113-9234	60-177	61-698	51-94
GGT (U/L)	-	8-22	16-22	14-16	10-19	11-22	10-18	11-20
WBC	4.0-10.0	4.1-13.4	8.3-12.1	6.9-12.7	4.9-9.2	4.8-9.7	4.5-9.2	4.8-8.0
SEGSA	0.6-4.5	0.6-5.8	1.4-5.1	3.9-6.5	0.9-2.8	1.1-3.1	0.9-2.3	0.6-1.4
BANDSA	0-1.0	0-0.2	0-0.8	0.2-0.9	0-0.1	0-0.1	0-0.1	0
LYMPHSA	1.8-7.5	2.7-8.3	2.0-8.1	2.6-4.7	2.1-6.8	2.9-6.9	3.5-7.2	4.1-7.0
EOSA	0-2.0	0-1.8	0	0	0-0.7	0-1.4	0-0.5	0-0.2
MONOSA	0.1-0.7	0-0.9	0-0.7	0.1-0.6	0-0.5	0.1-0.5	0-0.3	0-0.2
BASOA	Rare	0-0.1	0	0-0.1	0-0.2	0-0.2	0-0.1	0
PCV (%)	24.0-40.0	33.8-54.1	50.7-71.3	53.5-57.4	39.3-56.5	39.3-47.2	41.4-46.8	42.4-43.7
HGB (g/L)	8.0-14.0	10.7-19.5	19.1-25.5	21.3-23.0	13.4-20.2	15.2-18.5	15.1-16.8	15.5-16.5
RBC	5.0-9.0	7.9-14.1	11.8-17.9	13.3-15.8	9.6-12.9	10.0-11.7	10.4-11.8	10.8-11.7

Table B.6.8.2.10-7: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Clinical biochemical and haematological measurements (min-max values) for heifers given MON-0139

Parameter (unit)	Ref.[1,2]	Control[3]	Death on day 6 or 7		Surviving heifers			
			Sampled 6-14 h before death (3 heifers)	Sampled 34-38 h before death (2 heifers)	Signs of intoxication observed (5 heifers)		No signs of intoxication observed (3 heifers)	
					Days 2, 6, 8	Days 14 & 21	Days 2, 6, 8	Days 14 & 21
MCV (fL)	40.0-60.0	36.0-49.5	40.5-48.0	37.0-40.5	39.0-44.5	37.5-43.5	38.0-40.5	36.0-39.5
MCH (pg)	11.0-17.8	10.6-17.9	14.2-16.2	14.6-16.0	13.5-16.7	14.7-16.8	13.1-14.9	13.2-15.3
MCHC (g/dL)	30.0-36.0	27.9-40.0	35.8-37.7	39.8-40.1	31.5-39.6	34.0-41.7	34.8-37.7	35.6-38.7
STP (g/dL)	5.9-8.6	5.7-9.5	7.4-9.6	7.9-7.9	6.4-9.0	6.1-7.8	6.2-7.6	5.9-7.3

[1] Mitruka, BM and Rawnsley, HM: Clinical Biochemical and Haematological Reference Values in Normal Experimental Animals. Masson Publishing USA, Inc., New York, 1977.
[2] Archer, RK and Jeffcott, LB (eds.): Comparative Clinical Haematology, Blackwell Scientific Publications, London, 1977.
[3] Control values were obtained by pooling all pre-treatment values (principal and untreated controls) plus all post-treatment values from untreated controls on a study wide basis (99 measurements for each parameter from 20 heifers).
[4] Cells x 10^3/µL
[5] Cells x 10^6/µL
BUN = Urea nitrogen, CREAT = Creatinine, GLU = Glucose, Na = Sodium, K = Potassium, Ca = Calcium, P = Inorganic phosphorus, Mg = Magnesium, GOT = Glutamic oxaloacetic transaminase, LDH = Lactic dehydrogenase, SACH = Cholinesterase, ALKP = Alkaline phosphatase, CKN = Creatine phosphokinase, GGT = Gamma-glutamyltransferase, WBC = Total leukocyte count, SEGSA = Segmented neutrophil count, BANDSA = Band neutrophil count, LYMPHSA = Lymphocyte count, EOSA = Eosinophil count, MONOSA = Monocyte count, BASOA = Basophil count, PCV = Microhematocrit, HGB = Haemoglobin concentration, RBC = Erythrocyte count, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular hemoglobin concentration, STP = Total protein

Study conclusion

Exposure of Brahman-cross heifers for seven consecutive days to MON-0139 resulted in clinical signs of toxicity at doses of 830 mg/kg bw/day and above which included loss of appetite and diarrhoea. Mortality was observed at 1290 and 2000 mg/kg bw/day only. In addition, nervous system effects consistent with overt acute toxicity and impending death (head tremors, convulsions, ataxia, etc.) were observed at 2000 mg/kg bw/day. Changes in several haematological parameters observed at 1290 mg/kg bw/day and above may have been secondary to fluid and blood volume alterations resulting from the diarrhoea. Serum chemistry changes and histopathologic lesions indicative of renal damage were observed at 1290 mg/kg bw/day. The no-observable-effect level (NOEL) under the conditions of this study was considered to be 540 mg/kg bw/day.

Assessment and conclusion by applicant:

An aqueous solution of the isopropylamine salt of glyphosate (MON-0139) was administered on seven consecutive days by ruminal intubation to four groups of three female cattle (heifers) at 0, 540, 830, 1290 and 2000 mg/kg bw/day. Water was used as a vehicle to give a constant dosing volume of 500 mL. Four concurrent negative controls groups of two heifers each were sham treated with water. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology were assessed during a post-treatment observation period of 14 days.

Clinical signs of toxicity occurred at doses of 830 mg/kg bw/day and above, including loss of appetite and diarrhoea. Mortality occurred at doses of 1290 and 2000 mg/kg bw/day. Nervous system effects (head tremors, convulsions, ataxia, etc.) were observed at 2000 mg/kg bw/day prior to death.

At the two upper dose levels, haematological changes (haemoconcentration) probably resulted from diarrhoea. Serum chemistry changes and histopathologic lesions indicative of renal damage were observed at 1290 mg/kg bw/day. An increase in ASAT, creatine phosphokinase and lactate dehydrogenase activity suggests rather muscle than liver damage since there were no histological liver findings. Muscle damage could result from convulsions or prolonged sternal recumbency. Some further biochemical findings (blood urea nitrogen, creatinine) could be well due to dehydration but, in particular in the presence of histological kidney lesions, might also provide evidence of renal function impairment.

The statistical calculations in this study were based on very small group sizes of three treated and two negative control animals. Histopathology was confined to slides prepared for liver, kidney and tissues with gross lesions obtained from animals receiving doses of 1290 and 830 mg/kg bw/day and from 6 of the 8 control heifers. Limited data on organ weights was provided as kidney or liver to brain ratios only.

The study is considered acceptable despite the low number of animals per dose group. The quality of the study is suitable to provide additional information about subacute oral toxicity in a ruminant species. The lowest dose of 540 mg/kg bw/day can be considered a NOAEL for toxic effects in this study. Subacute oral toxicity of the isopropylamine salt of glyphosate in young cattle (heifers) was low. Forage application rate for grazing cattle to receive a dose equivalent to the NOAEL is estimated to be 77 pounds per acre (86 kg/ha), an unrealistically high use rate.

Assessment and conclusion by RMS:

It is agreed with the conclusion of the applicant that no adverse effects were observed at 540 mg/kg bw/day. However, the current RMS considers the number of animals to be too low to derive any meaningful NOAEL.

During the previous evaluation in the RAR (2015), the study was considered acceptable with a NOAEL of 540 mg/kg bw/day although the low number of animals was also noted.

B.6.8.2.11. In vivo/ ex vivo irritating effect of glyphosate, surfactant and roundup on stomach and small intestine

Data point:	CA 5.8.2/011
Report author	[redacted]
Report year	1987
Report title	Irritating effect of Glyphosate, Surfactant and Roundup on Stomach and small Intestine in Dogs
Report No	2309496
Document No	Not reported
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities.
Acceptability/Reliability:	Conclusion GRG: Supportive, Category 2a Conclusion AGG: Some limitations were noted including a lack of information on the purity of the test material and stability of the test compound. In addition, the acclimation period was not reported and the number of air changes was not included in the study report. Overall, the study is considered to be fit-for-purpose to evaluate the irritating effect of glyphosate on small intestines. Therefore, the study is

	acceptable but with restrictions (reliable with restrictions)

Full summary

This study evaluated the irritating effect of Roundup® (herbicide consisting of isopropylamine salt of glyphosate at 41%, surfactant MON-0818 at 15%, and water) and its ingredients on mucosa of fasted dogs. The irritating effects of Roundup, glyphosate and surfactant on gastric and small intestinal mucosa were tested *in vivo* using Beagle dogs. Histopathological findings were compared with effects caused by 0.25 N hydrochloric acid as positive control agent and with unchanged tissue samples. All test substances were applied directly to mucosae of anaesthetized dogs.

Direct application of Roundup, glyphosate acid, and surfactant caused only mild damage to the stomach and intestine. The severity of the damage was equivalent to that caused by 0.25 N hydrochloric acid. The test materials tended to affect the small intestine more than the stomach. Roundup and its surfactant caused decomposition of the tip of villi in the small intestine. Roundup, glyphosate and surfactant caused oedema and congestion of top mucosal layers and degeneration of epithelium in the stomach.

The oedema and ablation of epithelium were considered to have resulted from interstitial exudation due to congestion-caused microcirculatory disorder. As for the small intestine, in particular, congestion of villi produced oedema in the space between absorptive epithelium and basal membrane, which subsequently pushed up the epithelium to the extent that it might be ablated and detached.

These effects were more severe with the Roundup formulation than with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid. Clinical, autopsy and experimental evidence indicate a potential for gastrointestinal damage from glyphosate components of glyphosate formulations, but the frequency of severe injury appears to be low. Irritation was more severe with the Roundup formulation than with either the IPA salt or the surfactant alone. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.

The IPA salt of glyphosate, Roundup herbicide (41% IPA) and the surfactant MON-0818 (15% of which is contained in Roundup) and 0.25 N hydrochloric acid solution (control) were directly administered on the gastric and small intestinal mucosa of fasted male beagle dogs. The specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues. Direct application of Roundup® herbicide, and the surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup formulation than. with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid. The results suggest that these substances could significantly contribute to the toxicity of glyphosate products.

I. MATERIALS AND METHODS

A. MATERIALS

Test material 1:	Undilute Roundup Formulation
Identification:	41% isopropylamine (IPA) salt of glyphosate
Lot #:	Oda B6E20 88.10
Purity:	Not reported
Stability of test compound:	Not reported
Test material 2:	Isopropylamine salt of glyphosate
Identification:	Isopropylamine salt of glyphosate (MON139)
Lot #:	LIRT09023
Purity:	63.2% assay
Stability of test compound:	Not reported

Test material 3:

Identification:	Undilute Surfactant (MON-0818)
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported

Positive control:

Identification:	0.25 N hydrochloric acid
Source:	Kokusan Kagaku Co. Ltd.
Purity:	Not reported
Stability of test compound:	Not reported
Vehicle and/ or positive control:	Distilled water

Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approximately 15 months
Sex/Number:	Male, one test group consisting of 4to 8 animals
Body weight:	12-15 kg
Acclimation period:	Not reported
Diet/Feed:	DS (Oriental Yeast Co. Ltd.) , *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Stainless steel two-stage cages (Watering System Co.)
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 55 ± 10 % Air changes: not reported Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

Administration of test solutions

Roundup was applied undiluted. IPA salt of glyphosate and surfactant were diluted with distilled water to 41 % and 15 % respectively for application. The positive control 6N hydrochloric acid was diluted to 0.25 N solution with distilled water.

Beagle dogs, fasted for a day, were anesthetized with Ketamine and Skisamesonium, and the abdominal incision was performed. The stomach was cut open along the greater curvature with a cautery knife. The anterior wall of the stomach was everted to expose the mucosal tissue. In order to prevent the reflex of bile, etc., cheese cloth was inserted into the oesophagus and the pylorus. Using a silk suture, the gastric mucosa was pulled up to stuff cheese cloth into the serosa side of the posterior wall , thus making two 3cm diameter depressions on the surface of the body of stomach. Into these concavities, the test materials were placed, and left for 30 minutes. Whenever the test material was spilled or reduced in volume for other reasons, a sufficient amount of the test material was added. The small intestine was ligatured at two positions which were 10 cm apart. When doing this, with silk sutures care was taken not to stop blood circulation in the area concerned. A Teflon-coated catheter was inserted into the intestinal duct for administration of test solutions. Each test article was left in the test portion of the intestinal duct for 30 minutes as in the case of the stomach. After the 30-minute application, the stomach was immediately washed with physiological saline and the treatment portion of the stomach was incised for collection. The small intestine was cut open to evert the inner mucosal lining and washed with physiological saline.

The test material-applied portions of stomach and small intestine were fixed with 10% formalin buffer and their specimens were prepared by a routine method of haematoxylin and eosin stain. These specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues.

II. RESULTS AND DISCUSSION

A. HISTOPATHOLOGY OF GASTRIC MUCOSA

The stomach had slight oedema on the mucosa, congestion of the mucosa and very slight degeneration of the epithelium, after Roundup was treated. Application of glyphosate and surfactant also caused similar changes. Glyphosate affected the stomach approximately the same way and to the same degree as Roundup. As for surfactant, congestion of the mucosa was slightly milder than that for Roundup, but other observations were similar. A 0.25 N hydrochloric acid brought the same changes as Roundup, though the oedema and congestion of mucosa were slightly milder than those for Roundup were, while the degeneration of epithelium was slightly more severe.

B. HISTOPATHOLOGY OF SMALL INTESTINE MUCOSA

Roundup caused congestion of villi, oedema of the furthest portion of villi, degeneration and ablation of absorptive epithelium and dilatation of crypts. Glyphosate and surfactant showed the similar changes, though surfactant had slightly milder effect in oedema and glyphosate and surfactant had slightly milder effect in congestion of villi than Roundup. Surfactant did not cause dilatation of crypts, which the other two test materials did. A 0.25 N hydrochloric acid was similar in effect to Roundup, though the severity of the changes brought by the hydrochloric acid was a little higher, or similar to that by Roundup.

There was no remarkable change in the mucosa of a normal stomach. The Roundup- treated stomach had slight oedema and congestion of mucosal layers and degeneration of epithelium. There were similar changes in similar severity observed in the stomach treated with glyphosate. Surfactant had slightly milder effect in congestion of mucosa than Roundup , but there were no other differences between surfactant and Roundup as to the changes and their severity. The 0.25 N hydrochloric acid applied stomach displayed damages on mucosa similar to those for Roundup.

There was no remarkable change in a normal intestine. Roundup treatment caused congestion of villi, oedema of villi, degeneration and ablation of epithelium and dilatation and crypts. The glyphosate treated intestine also showed similar changes to the same degree , though its congestion of villi was less severe than that for Roundup. As for surfactant, the same observations except dilatation of crypts were made, but the congestion and oedema of villi were slightly milder than those for Roundup. A 0.25 N hydrochloric acid showed the same changes as Roundup, but the severity of those changes was slightly higher than , or at least the same as that for Roundup. In some cases, decomposition of the tip of villi was observed in the intestines treated with Roundup or surfactant.

Table B.6.8.2.11-1: Irritating effect of Glyphosate, Surfactant and Roundup on Stomach and small Intestine in Dogs ([redacted], 1987): Histopathological findings

	Findings of stomach after treatment with				**Findings of intestine after treatment with**			
	Roundup n=5	**Glyphosate** n=5	**Surfactant** n=4	**HCl** n=4	**Roundup** n=8	**Glyphosate** n=8	**Surfactant** n=8	**HCl** n=5
	Congestion of mucosa				**Congestion of villi**			
None	-	-	2	1	-	-	1	-
Very slight	-	1	1	2	-	1	2	-
Slight	4	3	1	1	2	5	3	-
Moderate	1	1	-	-	5	2	2	4
Severe	-	-	-	-	1	-	-	1
	Oedema of mucosa				**Oedema of villi**			
None	-	-	-	-	-	-	-	-
Very slight	-	1	1	2	2	2	5	-
Slight	5	4	3	2	6	6	3	5
Moderate	-	-	-	-	-	-	-	-
Severe	-	-	-	-	-	-	-	-
	Degeneration of epithelium				**Degeneration and ablation of epithelium**			
None	-	-	-	-	-	-	-	-
Very slight	5	5	4	2	2	2	3	-
Slight	-	-	-	2	5	4	4	5

Moderate	-	-	-	-	1	2	1	-
Severe	-	-	-	-	-	-	-	-
					Dilatation of crypts			
None	-	-	-	-	3	1	8	4
Very slight	-	-	-	-	2	3	-	1
Slight	-	-	-	-	3	4	-	-
Moderate	-	-	-	-	-	-	-	-
Severe	-	-	-	-	-	-	-	-

n = number of animals

Study conclusion

Roundup, glyphosate and surfactant caused oedema and congestion of top mucosal layers and degeneration of epithelium in the stomach. Treatment of 0.25 N hydrochloric acid also caused the same changes, but the severity was similar to or slightly lower than that by Roundup. As for the intestinal treatment, the three test articles caused congestion of villi, oedema of tips of villi and degeneration and ablation of epithelium. In addition to these, glyphosate and Roundup treated tissues showed dilatation of crypts. A 0.25 N hydrochloric acid also showed the same changes, but the severity of those changes was slightly higher than, or at least the same as that for Roundup. The mucosal damage in the stomach and small intestine caused by treatment of Roundup, glyphosate of surfactant was only mild, equivalent to that caused by 0.25 N hydrochloric acid. The intestine was more severely damaged than the stomach in every case.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The 63.2% concentrate IPA salt of glyphosate, undiluted Roundup herbicide (41 % IPA, 15 % MON-0818 surfactant), the undiluted surfactant MON-0818, and 0.25 N hydrochloric acid solution (control) were directly administered on the gastric and small intestinal mucosa of fasted male beagle dogs. The specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues. Direct application of Roundup® herbicide, and the surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup formulation than with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.

The data are considered as supplemental information indicating that highly concentrated glyphosate IPA salt has irritating properties on the stomach and intestine of dogs, nevertheless has a lower impact than the undiluted Roundup herbicide.

Assessment and conclusion by RMS:

The conclusion made by the applicant is agreed with. Glyphosate, the surfactant and the formulation Roundup showed irritating properties on the stomach and small intestine. The strongest effect was observed with the formulation.

During the previous EU evaluation the study was not extensively evaluated. Instead reference was made to the original DAR in which it was concluded that glyphosate has irritating properties on the stomach.

B.6.8.2.12. Acute intraperitoneal toxicity study in rat

1. Information on the study

Data point:	CA 5.8.2/012

Report author	Anonymous
Report year	1980
Report title	Acute intraperitoneal toxicity of glyphosate in female rat
Report No	Not reported
Document No	Not reported.
Guidelines followed in study	None stated.
Deviations from current test guideline	No guidelines applicable for acute toxicity studies following intraperitoneal injection. However, compared to the 'standard' acute toxicity studies, the following deviations were noted: – Reporting of results is minimal. Substantial information is missing. – Non-fasted animals were dosed. – Bacterial or fungi infection appears to have occurred as peritonitis was observed at the end of the study.
Previous evaluation	Not retrievable in the previous RAR (2015); Supplementary in the DAR (1998).
GLP/Officially recognised testing facilities[1,2]	Not specified.
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 4a Route of exposure (intraperitoneal injection); deficiencies in reporting (only limited information on test material, test animals and results available). **Conclusion AGG:** The study is considered to be not acceptable due to the deviations noted above.

2. Full summary

The test substance, N-phosphono-methyl-glycine, was evaluated for its acute intraperitoneal toxicity potential, by exposing groups of ten Sprague-Dawley strain female rats to doses of 620, 760, 940, 1040 and 1260 mg/kg bw. The test substance was diluted in propylene-glycol in water.
Mortality occurred in the 4 highest dose groups. In the highest dose group (1260 mg/kg bw), 10/10 animals died. In the next lower dose group (1040 mg/kg bw), 8/10 animals died followed by 4/10 and 1/10 deceased animals at 940 mg/kg bw and 760 mg/kg bw, respectively. Transient dyspnoea due to string irritation of viscera was observed. In the deceased animals, superficial liver necrosis, punctiform bleedings of the peritoneum and exudatum between the pleura surfaces were seen. The extent of fibrophosis extending to the viscera was proportional to the time of survival. The late deaths were considered to be caused by peritonitis instead of test substance-induced toxicity.
The acute intraperitoneal LD_{50} was calculated to be 919 ± 42 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	N-phosphono-methyl-glycine
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported
Vehicle and/ or positive control:	Propylene glycol in water/ None

Test animals:

Species:	Rat
Strain:	Wistar
Source:	Not reported

Age:	Not reported
Sex:	Females
Weight at dosing:	190 - 210 g
Acclimation period:	Not reported
Diet/Food:	Normal laboratory rodent food (LATI), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Not reported
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 - 75 % Air changes: 8/hour Hours light/dark cycle: Not reported

B. STUDY DESIGN AND METHODS

In life dates: Not reported

Animal assignment and treatment:
The dosage of the test substance was 50% concentration of the test substance in 10% propylene-glycol in water.The dose levels are shown in the following table.

Table B.6.8.2.12-1: Acute intraperitoneal toxicity of glyphosate in female rat (Anonymous, 1980): Dose levels

Sex	No. of rats	Dose level (mg/kg bw)
Female	10	620
	10	760
	10	940
	10	1040
	10	1260

No details on observations for mortality and clinical signs of toxicity is available.

II. RESULTS AND DISCUSSION

A. MORTALITY
The occurrence of deaths is shown in the following table.

Table B.6.8.2.12-2: Acute intraperitoneal toxicity of glyphosate in female rat (Anonymous, 1980): Mortality

Sex	No. of rats	Dose level (mg/kg bw)	Survival time of dead animals (days)	Mortality Dead animals (%)
Female	10	620	all survived	0/10 (0)
	10	760	9	1/10 (10)
	10	940	7-13	4/10 (40)
	10	1040	6-11	8/10 (80)
	10	1260	5-10	10/10 (100)

B. CLINICAL OBSERVATIONS
No test substance related symptoms were observed. According to the autopsy findings the observed transient limb anemia and the dispnoe were probably due to the strong irritation of viscera caused by the i.p. injection.

C. BODY WEIGHT
No data on body weight available.

D. NECROPSY
In the dead animals, superficial liver necrosis, punctiform bleedings of the peritoneum, exudatum between the pleura surfaces were seen. The extent of fibrophosis extending to the viscera was proportional to the time of survival. The late deaths were considered to be caused by peritonitis instead of test substance induced toxicity.

Study conclusion
The acute intraperitoneal LD_{50} for the test substance N-phosphono-methyl-glycine was calculated to be 919 ± 42 mg/kg.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Acute intraperitoneal toxicity of glyphosate was tested in rats. There is no OECD guideline available for this endpoint. As to be expected, toxicity was higher by this route as compared to oral, dermal or inhalation exposure. Due to the route of exposure and deficiencies in reporting, this study provides only supplementary information. Based on the study results, the acute intraperitoneal LD_{50} is 919 mg/kg bw in female rats.

Assessment and conclusion by RMS:
No guideline is available to address acute toxicity after intraperitoneal injection and this is not a data requirement either. However, compared to other OECD guidelines addressing acute toxicity, several deviations were noted (see above). Overall, the study is not considered acceptable and therefore the RMS did not derive LD_{50} values. In contrast to what the applicant stated, the study is not retrievable from the previous evaluation (RAR, 2015), however, the study was considered supplementary only in the first evaluation (DAR, 1998).

B.6.8.2.13. Acute intraperitoneal toxicity study in rat

1. Information on the study

Data point:	CA 5.8.2/013
Report author	[redacted]
Report year	1978
Report title	Acute toxicity study (intraperitoneal injection) of CP67573 on the rats
Report No	[redacted]-79-109
Document No	Not reported
Guidelines followed in study	None stated
Deviations from current test guideline	No guidelines applicable for acute toxicity studies following intraperitoneal injection. However, compared to the 'standard' acute toxicity studies, the following deviations were noted: – LD_{50} calculations based on the mortality after 7 days, not 14 days. Due to further deaths between 7 and 14 days after administration, the LD_{50} after 14 days would be lower. – Except for mortality, no results are shown in the study report. – Overall, reporting of results is minimal. Substantial information is missing.
Previous evaluation	Not retrievable in the previous RAR (2015); Supplementary in the DAR (1998).
GLP/Officially recognised testing facilities[1,2]	No
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 3a, Route of exposure (intraperitoneal injection); body weights not reported. **Conclusion AGG:** The study is considered to be no acceptable due to the deviations noted above.

2. Full summary

The test substance CP67573 was evaluated for its acute intraperitoneal toxicity potential by a single injection to Wistar rats (10 animals/group/sex). The test substance was applied at a dose of 182, 255, 357, 422 and 500 mg/kg bw to males and 255, 357, 500, 700, 980 and 1372 mg/kg bw to females. The animals were observed for a period of 14 days. The behavioural and pathological findings suggest that the test substance acts on the central nervous system and digestive organs. The acute intraperitoneal LD_{50} in male and female rats after seven days was calculated to be 281 and 467 mg/kg bw, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	CP67573
Description:	White powder
Lot/Batch #:	Not specified
Purity:	98.4 % glyphosate
Stability of test compound:	Not specified
Vehicle and/ or positive control:	0.5 % tragant solution

Test animals:

Species:	Rat
Strain:	Wistar-Imamichi
Source:	[redacted] (not further specified)
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	Male: 95 - 102 g; female: 94 - 100 g
Acclimation period:	Not specified
Diet/Food:	Fumabashi Mojyo Co., Ltd. chow, *ad libitum*
Water:	Drinking water, *ad libitum*
Housing:	Individual
Environmental conditions:	Temperature: 24 ± 1 °C Humidity: 50 - 60 % Air changes: 12/hours Photocycle: 14 hours light/10 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: not specified

Animal assignment and treatment

The test substance suspended in 0.5% tragant solution was prepared just prior to use in varying concentrations to maintain a dose volume of 0.5 mL/100 g bw. The dose levels are shown in the following table below.

Table B.6.8.2.13-1: Acute toxicity study (intraperitoneal injection) of CP67573 on the rats ([redacted], 1978): Dose levels

Sex	No. of rats	Dose level (mg/kg bw)
Male	10	182
	10	255

Table B.6.8.2.13-1: Acute toxicity study (intraperitoneal injection) of CP67573 on the rats ([redacted], 1978): Dose levels

Sex	No. of rats	Dose level (mg/kg bw)
	10	357
	10	422
	10	500
Female	10	255
	10	357
	10	500
	10	700
	10	980
	10	1372

Observations for mortality and clinical signs of toxicity were made during the 14-day post-exposure period. Body weights of individual rats were measured on Day 0, 7 and 14. All rats were subjected to a grosspathological examination. The LD_{50} and 95 % confidence limits were calculated by Lithchfield-Wilcoxon method used mortality of Day 7.

II. RESULTS AND DISCUSSION

A. MORTALITY

The occurrence of deaths is shown in the following table.

Table B.6.8.2.13-2: Acute toxicity study (intraperitoneal injection) of CP67573 on the rats ([redacted], 1978): Mortality

Sex	No. of rats	Dose level (mg/kg bw)	Day										Mortality (%)	
			1			2	3	4	5	6	7	8-14	Days 0-7*	
			1 h	6 h	24 h									
Male	10	182	0	0	0	0	0	0	0	0	0	0	0/10	(0)
	10	255	0	0	0	0	2	1	0	1	0	0	4/10	(40)
	10	357	0	0	0	1	1	2	2	0	1	2	7/10	(70)
	10	422	0	0	0	3	4	1	0	0	0	2	8/10	(80)
	10	500	0	0	0	2	6	2	0	0	0	0	10/10	(100)
Female	10	255	0	0	0	0	0	0	0	0	0	0	0/10	(0)
	10	357	0	0	0	0	0	1	1	0	0	4	2/10	(20)
	10	500	0	0	0	1	4	0	1	0	0	1	6/10	(60)
	10	700	0	0	0	1	2	3	1	2	0	1	9/10	(90)
	10	980	0	5	0	3	0	0	0	1	0	1	9/10	(90)
	10	1372	0	10	0	0	0	0	0	0	0	0	10/10	(100)

* The LD_{50} was calculated after 7 days, due to further deaths in the second week the 14-day LD_{50} would have been lower in both cases.

B. CLINICAL OBSERVATIONS

Thirteen minutes after administration of CP67573, ventral posture, decrease of spontaneous activity, piloerection, salivation and decrease in skin temperature were observed in all animals of both sex. These signs were not observed on the following morning in the lowest dose groups of males and females (182 mg/kg bw and 255 mg/kg bw, respectively). In other dose groups, these symptoms were observed for 36 to 48 hours.
In dead animals disappearance of righting reflex and coma was observed. Animals died in ventral posture.

C. BODY WEIGHT

Although it is stated that body weights were recorded, results are not shown in the study report.

D. NECROPSY

Gross pathological findings present in deceased animals were: increase in circumference of the abdomen; ascites, hyperplasia in intestine wall; adhesion, transformation and slight hypertrophy of liver lobes (with hypertrophy of

peritoneum); haemorrhage of mucosa in fundamental stomach; spot haemorrhage in thymus and peyr patches; hyperemia in adrenals.
The signs observed at autopsy of the survivors were as follows: adhesion, slight hypertrophy and transformation of liver lobes and adhesion of stomach and pancreas, hyperplasia of spleen capsule in all animals. The animals that increased in circumference of the abdomen revealed a decrease in body weights and hypertrophy of cecum.
Since the changes of the liver were due to hyperplasia of peritoneum induced by mechanophysical stimulation of injected suspension, the observed findings in the liver were not attributed to a test substance specific induced toxicity.

Study conclusion
The acute intraperitoneal LD_{50} after 7 days for the test substance CP67573 in males and females was calculated to be 281 mg/kg bw and 467 mg/kg bw, respectively. The 95 % confidence limits were 210 - 340 mg/kg bw and 383 - 592 mg/kg bw for males and females, respectively.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Acute intraperitoneal toxicity of glyphosate was tested in rats. There is no OECD guideline available for this endpoint. As to be expected, toxicity was higher by this route as compared to oral, dermal or inhalation exposure. Due to the route of exposure, this study provides only supplementary information. Based on the study results, the acute intraperitoneal LD_{50} is 281 mg/kg bw and 467 mg/kg bw in male and female rats, respectively.

Assessment and conclusion by RMS:
No guideline is available to address acute toxicity after intraperitoneal injection and this is not a data requirement either. However, compared to other OECD guidelines addressing acute toxicity, several deviations were noted (see above). Overall, the study is not considered acceptable and therefore the RMS did not derive LD_{50} values. In contrast to what the applicant stated, the study is not retrievable from the previous evaluation (RAR, 2015), however, the study was considered supplementary only in the first evaluation (DAR, 1998).

B.6.8.2.14. In vitro dermal absorption study through rabbit skin

Data point	CA 5.3.3 (submitted as KCA 5.8.2/014)
Report author	[redacted]
Report year	2012
Report title	Glyphosate acid - *In Vitro* Absorption through Abraded Rabbit Skin using [^{14}C]-glyphosate
Report No	JV2182-REG
Document No	Not reported
Guidelines followed in study	OECD 428 (2004)
Deviations from current test guideline (OECD 428, 2004)	Receptor fluid solubility was not tested.
Previous evaluation	Yes, accepted in RAR, but considered supplementary only (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered supplementary only.

The purpose of this study was to determine the *in vitro* percutaneous absorption of glyphosate acid through abraded rabbit skin following a 6-hour exposure period and subsequent 18 hour monitoring period. This study was designed to assess the potential dermal penetration of test material through rabbit skin and will be of use in estimating the systemic dose achieved in a previous *in vivo* rabbit dermal toxicity study (see CA 5.3.7/01, [redacted], 1982). Therefore, the application rate and exposure conditions used in this study were calculated to be

equivalent to 5000 mg/kg bw/day as applied to rabbits in the *in vivo* dermal study (CA 5.3.7/01).

^{14}C-glyphosate was incorporated into a wet cake preparation prior to application. The preparations were applied as a paste to abraded rabbit skin membranes at a rate of 79.8 mg/cm^2 (corresponding to 48.3 mg glyphosate acid/cm^2) and left un-occluded for an exposure period of 6 hours, after which the skin surface was washed. The absorption process was followed by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout a total time-period of 24 hours. The distribution of glyphosate within the test system and a 24-hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).

The results of this *in vitro* study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow. The vast majority of glyphosate will be washed off during normal washing procedures. The mean total amount of glyphosate absorbed after 24 hours was 2.42 % of the applied dose. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 %.

Materials and methods

A. Materials

1. Test materials:

a) Non radio-labelled test substance:

Identification:	MON 77973 (glyphosate acid)
Description:	White wet cake
Lot/Batch #:	GLP-1103-21149-T
Chemical purity:	85.14 % as glyphosate acid (purity: 95.93 %)
Stability of test compound:	Expiry date: 2012-03-09

b) Analytical reference standard:

Identification:	Glyphosate acid
Lot/Batch #:	GLP-0810-1915-A
Chemical purity:	Not reported

c) Radio-labelled test substance

Identification:	^{14}C-glyphosate (as glyphosate acid) [phosphonomethylene-^{14}C]-Glyphosate
Lot/Batch #:	4675JJN002-1
Radiochemical purity:	96.7 % (confirmed by analysis)
Specific activity:	48 mCi/mmol; 1776 MBq/mmol; 2523µCi/mL; 9.35 MBq/mL

2. Test skin source:

Species:	Rabbit
Strain:	New Zealand White Albino
Source:	Harlan
Age:	At least 12 weeks
Type:	Complete pelt

B: Study design and methods

Preparation of skin samples:
Skin pelts from New Zealand White albino rabbits at least 12 weeks old were obtained from Harlan. The skin samples were transported on cold blocks and were stored on arrival at -20 °C, the day after sacrifice. The skin samples arrived clipped and excised and were examined for scars and blemishes. Any extraneous subcutaneous tissue was removed after defrosting and the pelts clipped further if necessary. The pelts were given an

identifying number and individually stored frozen, at approximately -20 °C, on aluminium foil until required for use.

Test substance preparation
The doses were prepared, to mimic as closely as possible a 5000 mg/kg bw dose from a previous rabbit *in vivo* study (CA 5.3.7/01, [redacted], 1982). The dose equivalency was calculated on a dose per unit area of skin basis using an average *in vivo* rabbit weight of 2.78 kg. The doses were prepared as close to the time of application as was practicable.

Radioactive stock solution of ^{14}C-glyphosate
The radiolabelled ^{14}C-glyphosate was supplied as a solution in water.

Trial preparation of the radiolabelled glyphosate acid
Glyphosate acid trial preparation was prepared using the method described below, with the exception that a different volume or smaller amounts of radioactivity or unlabelled material were used, where applicable. Three individual vials were prepared as part of the trial preparation, to assess dosing methodology. The paste like composition of the dose preparation was investigated to ensure that it visually provided good skin contact during application to the membranes.

Preparation of radiolabelled glyphosate acid
Firstly 8008 mg of non-labelled glyphosate wet cake was added to a vial, followed by 4162 µL of radiolabelled glyphosate stock solution, providing a nominal 3.85 mg of glyphosate (40 MBq) radioactivity. 5 mL of water was then added and the preparation mixed thoroughly. The preparation was then freeze dried to remove the water added and the water present in the wet cake. When dry, the glyphosate wet cake preparation was then weighed to confirm the removal of all the water. Approximately 521 mg of the dried wet cake preparation was then added to 8 individual vials together with approximately 300 µL of saline to each vial to create a paste. A final weight of each vial was recorded and the preparation was thoroughly mixed with a spatula into a paste before dosing.

Preparation of non-labelled glyphosate acid
To demonstrate that the dose preparations have a close contact during the application procedure, an additional dose preparation without radiolabel was prepared according to the procedure described above.

Analyses of dose preparations
The radioactivity content of the stock solution was determined by liquid scintillation counting (LSC) analyses of sub-samples of solvent dilutions. The radiochemical purity of the radiolabelled stock solution of the test substance was determined by thin layer chromatography (TLC) using unlabelled test substance as reference standard.
The radioactivity content and homogeneity of the dose preparations were checked by LSC analyses. The radiochemical purity and stability was measured by TLC analyses.

Preparation of diffusion cells
The skin membranes were placed in static glass diffusion cells providing an exposure area of 2.54 cm^2 of skin. The cells had a receptor volume of approximately 4.5 mL.
An integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. Non-abraded membranes with a resistance of 1.5 - 5 kΩ were considered having a normal integrity and used for the skin abrasion. Rabbit skin was abraded using a blunt spatula drawn over the skin area approximately six to eight times, in the form of a grid, in order to mimic 'Draize' abrasion as conducted in the *in vivo* study (CA 5.3.7/01, [redacted], 1982). After the abrasion a further integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. For abraded skin samples membranes with ER values in the range of 0.7 – 1.0 kΩ were selected for the study.
Cells were selected such that the application rate was represented by eight intact skin samples from five different animals. Physiological saline was chosen as receptor fluid. The skin surface temperature was maintained at 32 ± 1 °C using a water bath.

Test substance application and sampling
Prior to dosing a pre-treatment sample of 500 µL was taken from each diffusion cell, and replaced by an equal amount of fresh receptor fluid.
Each dose formulation was applied to the abraded skin membrane as a dried glyphosate acid wet cake paste and

spread over the skin surface using a spatula. The weight of each individual preparation and spatula were recorded before and after dosing to allow the applied dose to be calculated.

Each dose was applied at the nominal rate of 79.8 mL/cm^2 exposed skin area (202.8 mg/cell), corresponding to 48.3 mg glyphosate/cm^2. The applications were left un-occluded for 24 hours.
Receptor fluid samples (500 µL) were taken by an auto-sampler at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. After each sampling the removed amount of receptor fluid was replaced by an equal amount of fresh receptor fluid.
After the 6-hour sampling, the skin samples were washed by gently swabbing the application site application site with at least three natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. Two further sponges, pre-wetted with water, were used to further swab the surface.

Terminal procedures
After the last sampling, 24 hours after application the remaining receptor fluid was discarded. The receptor chamber was rinsed with receptor fluid that was also discarded.
The donor chamber was carefully removed and the underside wiped with a single natural sponge, pre-wetted with 3 % Teepol L® in water, which was added to the wash sponges. The donor chamber was washed with deionised water and a sample was taken for LSC analysis.
The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The skin surface was washed with further sponges pre-wetted with water. All the sponges were combined and digested in Soluene 350® and made up to a recorded volume. A sample was taken for analysis.

Due to the fragility of the abraded skin samples tape stripping could not be performed. Instead a heat separation technique was used to separate the epidermis from the dermis:

The skin was carefully removed from the receptor chamber and the flange area cut away and digested in Soluene 350® and aliquots taken for analysis by LSC.

The remaining skin disc was placed dermis side down, on cling film. A second piece of cling film was then used to cover the epidermis side. A 200 g weight was placed in a water bath at 65 ºC for an hour prior to use. The weight was placed onto the epidermal surface with moderate pressure for approximately 90 seconds. The epidermis was peeled away from the dermis using forceps. The dermis was digested in Soluene 350® and aliquots taken for analysis by LSC. The epidermis was digested in Soluene 350® and the whole sample analysed by LSC.

Analysis of samples
The radiochemical purity and stability of the ^{14}C-glyphosate preparations was determined by TLC using silica gel plates and methanol:water:acetic acid (6 : 3 : 0.5, v/v/v). Radioactivity on the TLC plates was measured using a Packard Instant Imager (SOP E003). Unlabelled material was visualised under UV light at 254 nm.
For visualising the test material on the TLC plates a 2 % ninhydrine solution in acetone was used. In addition, for analyses of dose preparations K2 cellulose plates and a revised solvent system (methanol: water:acetic acid (8 : 1.5 : 0.5, v/v/v) was used.

Liquid samples of the receptor fluid, washing solutions, digested wash sponges, and digested dermis and epidermis were measured by LSC using a Packard 3100 TR LSC counter and Goldstar as scintillation fluid.
Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of glyphosate in the receptor solution in terms of µg/cm^2. The amounts absorbed, rates of absorption (µg/cm^2/h) and ‘percentage of dose absorbed’ were calculated. Membranes with absorption profiles that indicate membrane damage during the course of the experiment have been excluded from calculations. The results of the mass balance and distribution determinations were expressed in terms of amount absorbed and ‘percentage of applied dose.
The absorbed dose was considered the glyphosate detected in the receptor fluid, while the potentially biologically available proportion of the dose was regarded as the sum of absorbed dose and the amount recovered from the dermis. The test material removed from the surface of the epidermis by the washing procedure, as well as the glyphosate recovered from the epidermis at the end of the exposure was considered unabsorbed.

Results

A. ANALYSES OF THE ^{14}C-GLYPHOSATE STOCK SOLUTION

TLC analysis of the ^{14}C-glyphosate stock solution confirmed a radiochemical purity of greater than 95 %. LSC analysis revealed a radioactivity content of 72.1 MBq, equivalent to a concentration of 0.924 mg/mL. The stock solution was homogeneous with a 1.31 % deviation between the replicates.

B. ANALYSES OF DOSE PREPARATIONS

LSC analyses confirmed the mean application rate to be 48.3 mg glyphosate/cm^2. The dose preparations had low variability between the replicates analysed (1.66 – 6.26 %) and, considering the physical nature of the preparation, the dose preparations were considered to have acceptable homogeneity.

C. MEMBRANE INTEGRITY CHECK

Based on the ER measurements eight cells with abraded skin samples were selected for the absorption study.

D. DERMAL ABSORPTION OF GLYPHOSATE

Absorption profiles were assessed from eight abraded skin samples. Since one skin sample showed an atypical absorption profile, this was excluded from the calculation of means and SD.

The determined distribution of radioactivity are summarised in the table below.

Table B.6.8.2.14-1: Glyphosate acid - *In Vitro* Absorption through Abraded Rabbit Skin using [^{14}C]-glyphosate ([redacted], 2012): Summary of results for dermal absorption of ^{14}C-glyphosate

Dose preparation		
Applied dose "wet cake" [mg/cm^2]	79.8	
Applied dose glyphosate [mg/cm^2]	48.3	
Number of cells assessed	7	
	Distribution of radioactivity (mean values ± SD**)**	
	µg/cm^2	% of applied dose
Surface compartment		
Dermis (after heat separation)	118 ± 19.4	0.243 ± 0.040
Skin wash at 6 hours	42,802 ± 3008	87.9 ± 6.30
Skin wash at 24 hours	1159 ± 1224	2.38 ± 2.51
Donor chamber	59.2 ± 56.9	0.121 ± 0.117
Receptor compartment		
Receptor fluid 0 – 24 h	1177 ± 244	2.42 ± 0.503
Total absorbed*	**1177**	**2.42**
Epidermis (after heat separation)	20.1 ± 9.97	0.041 ± 0.020
Flange area	132 ± 68.6	0.270 ± 0.141
Total potentially absorbable**	**1295**	**2.663**
Total recovery	**45,468 ± 2096**	**93.3 ± 4.46**
Absorption rates 0 – 24 h [µg/cm^2/h]	53.1 ± 10.2	-

SD: Standard deviation; * Amount in receptor fluid; ** Total potentially absorbable = total absorbed + remaining dermis (after heat separation)

The total recovery of the individual cells was in the range of 87.3 % to 98.2 %, with an overall mean recovery of 93.3 % of applied dose.

The majority of the applied glyphosate acid (mean 87.9 %) was washed off the skin at 6 hours, with a further 2.38 % washed off at 24 hours. A small proportion (0.041 %) of the dose applied was recovered from the epidermis, with 0.243 % remaining in the dermis.

The mean rate of absorption of glyphosate acid between 0-1 hours was 47.0 µg/cm^2/h, which increased to 166 µg/cm^2/h between 1 – 4 hours. The mean absorption rate subsequently slowed to 72.3 µg/cm²/h between 4-10 hours and declined further to 13.3 µg/cm²/h for the remainder of the absorption period (10 – 24 hours). The overall absorption rate (0 – 24 hours) was 53.1 µg/cm^2/h.

The mean amount of glyphosate acid that penetrated abraded rabbit skin into the receptor fluid over the entire 24-hour experimental period was 1177 µg/cm^2, corresponding to 2.42 % of the applied dose.

Considering that the amount found in the remaining dermis after 24 h was potentially available and could further penetrate through the skin, the total amount of glyphosate potentially available was 2.66 % of the applied dose.

Assessment and conclusion by applicant:
In this study, the *in vitro* percutaneous absorption of glyphosate acid through abraded rabbit skin was assessed following a 6-hour exposure period and a subsequent 18 hour monitoring period. The study was conducted according to OECD 428 (2004) and in compliance with GLP regulations. There were no deviations from OECD 428 (2004). Therefore, the study was considered valid.
The results of this *in vitro* study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow. The vast majority of glyphosate will be washed off during normal washing procedures. The mean total amount absorbed after 24 hours was 2.42 % of the applied dose. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 % of the applied dose. Applying this to the repeated dose rabbit dermal toxicity NOAEL of 5000 mg/kg bw/day in [redacted] (CA 5.3.3/008) a systemic NOAEL in rabbit repeated dose studies is determined to be 133 mg/kg bw/day.

Assessment and conclusion by RMS:
The RMS largely agrees with the assessment of the applicant. The study is considered valid and in accordance with OECD 428 except for the fact that the receptor fluid solubility was not assessed in the frame of this study.

Although the study setup is considered acceptable, the study itself is only of limited value for risk assessment. Dermal absorption of an active ingredient must always be determined or estimated for a certain formulation under evaluation. Exposure to glyphosate present in a "dried wet cake" does neither reflect real exposure conditions of operators, bystanders or workers to liquid concentrates or spray dilutions nor a possible impact of co-formulants. The EFSA guidance on dermal absorption 2017 has not been used in the assessment, however, this more recent guidance is not further considered as the study is considered supplementary only.

The assessment of the study is in line with the previous evaluation (RAR, 2015).

B.6.8.2.15. Public literature – in vitro screening using human-derived 3D bioengineerd liver and cardiac organoids

Data point:	CA 5.8.2/015
Report author	Forsythe, S.D. *et al.*
Report year	2018
Report title	Environmental Toxin Screening Using Human-Derived 3D Bioengineered Liver and Cardiac Organoids
Document No	doi: 10.3389/fpubh.2018.00103 ISSN: 2296-2565
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG:** Yes/Reliable with restrictions **Conclusion AGG:** The study is not considered reliable and/or relevant for evaluation (see "Assessment and conclusion by RMS" for details).

Full summary
In this study the toxicity of glyphosate for liver and cardiac organoids was investigated in the concentration range from 25 µM to 25 mM. The endpoints considered were cell viability, ATP activity and beating rate of the cardiomyocytes. Glyphosate was shown to reduce organoid integrity and viability at doses from 250 µM to

2.5 mM. The IC_{50} values based on ATP activity of liver and cardiac organoids were found to be 10.53 and 10.85 mM, respectively. When cardiac organoids were exposed to glyphosate at 0.25 mM a non-statistically significant effect was found on beating rate. Exposure to 2.5 mM for 2 days resulted in all organoids stopping beating.

I. MATERIALS AND METHODS

Test material

The test material was purchased from Sigma Aldrich. The purity of glyphosate were not reported.

Liver and cardiac cell sources, culture and organoid formation

For liver organoids, all cells used were commercially sourced, human primary cells. Hepatic stellate cells (HSCs) were expanded in culture for two passages before cryopreservation for use in organoid formation. During expansion, HSCs were cultured in 90 % high glucose DMEM and 10 % fetal bovine serum on a rat tail collagen I coating (10 g/cm^2) at 37 °C with 5 % CO_2. Primary human hepatocytes were thawed according to the manufacturer's instructions using hepatocyte thawing medium. Kupffer cells were also thawed following the manufacturer's instructions. After thawing, primary human hepatocytes were plated on collagen coated (10 g/cm^2) 6-well culture plates, using hepatocyte plating medium at a density of ~ 150,000 cells/cm^2. Cells were incubated at 37 °C with 5 % CO_2 for 4 hours before adding matrigel as an overlay. Following further incubation for 24 hours, fresh HCM medium was added.

For cardiac organoids, induced pluripotent stem cell-derived cardiomyocytes (iPSC CMs) were commercially sourced from Axiogenesis (cat. #COR.4U Cardiomyocytes). Human primary cardiac fibroblasts were commercially sourced from ScienCell (cat. #6330). Prior to organoid formation, iPSC CMs were cultured on tissue culture plastic for 48 hours in COR.4U medium until cells began beating spontaneously. At this point, iPSC CMs were harvested using trypsin-EDTA.

The liver cells were combined in a cell seeding mixture comprised of 90 % HCM medium, 10 % heat-inactivated fetal bovine serum, and rat tail collagen I (10 ng/µL). Liver organoids were produced with a mixture of 80 % hepatocytes, 10 % hepatic stellate cells, and 10 % Kupffer cells. Approximately 1,500 cells per 40 µL medium were used to form aggregates in each well of non-adherent, round-bottom, 96-well plates to produce spherical organoids. Cardiac organoids were produced similarly. IPSC CMs were suspended in cardiomyocyte maintenance medium. Fibroblasts were added as 10 % of the total cell number, and the volume was adjusted to reach a cell density of 10,000 cells/mL. 100 µL of cell suspension was pipetted into each well of non-adherent, round-bottom, 96-well plates to produce spherical organoids resulting in approximately 1,000 cells/organoid. Well plates were incubated and observed daily until organoid formation, and then immediately used in experiments.

Preparation of test item stock solutions

Glyphosate was dissolved in DI H_2O for environmental toxins to reach 50 mM. Serial dilutions were performed in media for each cell type until all concentrations were created at 2X final concentration. Glyphosate was assessed in the concentration range from 25 µM to 25 mM. Glyphosate (70.98 mM at 25°C H_2O) did not dissolve readily until constant stirring and heat was applied, it remained dissolved at 25 mM in DI H_2O with no precipitation after storage.

Live/dead staining

Organoids were isolated from 96-well low adhesion round bottom plates, suspended in Hystem hydrogel in a 20 µL construct, and placed into 12-well plates to immobilize organoids in a 3D extracellular matrix environment. Each concentration of glyphosate premixed at 1X concentration in media according to organoid type was added to individual wells and organoids were allowed to incubate under their respective conditions for 2 days at 37 °C with 5 % CO_2. Studies were performed using n = 5 or higher for all conditions. Medium was then removed and organoids were assessed by LIVE/DEAD® Viability/Cytotoxicity Kit assays. Specifically, 2.0 µM calcein AM and 4.0 µM ethidium homodimer in PBS was added to each well and was allowed to incubate for 1 hour. Imaging was then performed by macro-confocal microscopy and composite images were created with ethidium bromide red fluorescence representing dead nuclei and calcein AM green fluorescence representing live cells.

ATP activity assays

Glyphosate was added in a premix 2× concentration of 100 µL solution to each well of a 96-well plate containing an organoid with 100 µL of medium and allowed to incubate for 2 days at 37 °C with 5 % CO_2 with n = 6 or higher. Medium was then removed from each well leaving 100 µL of media remaining along with the organoid. Next, 100 µL of Cell-Titer Glo Luminescent Cell Viability Assay solution was added to each well along with

100 µL added to 100 µL DMEM in a Costar Black Polystrene 96-well assay plate and allowed to incubate for 10 minutes at room temperature shielded from light. The entire contents of the wells containing organoids were then added to the Costar Black Polystrene 96-well assay plate wells and the contents were read on a Vertias Microplate Luminometer using default settings. Values were then averaged among the different groups and graphed for analysis using Graph Pad Prism© software.

Heart beat assay
Fully formed cardiac organoids in the wells of a 96-well non-adhesive round bottom plate were placed under a Leica DMIL LED microscope to allow for the recording of natural beat rates in 20 s videos (n = 3). The plate was then returned to the 37 °C, 5 % CO_2 incubator for 5 minutes to ensure that organoids did not experience significant temperature decreases, which can detrimentally impact beating rates. The process was repeated until all experimental subject organoids under the test compound concentrations described above, but at varying time points, had been recorded. Glyphosate was added as 2 × 100 µL concentration to each well of a 96-well low adhesion round bottom plate containing 100 µL of normal medium with a cardiac spheroid, and allowed to incubate for 30 minutes at 37 °C. The plate was then recorded, 3 organoids at a time, in the process listed above until all organoids were recorded and the plate was returned to the incubator. The process was then repeated 24 and 48 hours later. The 20 s videos were then analyzed by counting the beats for each video and multiplied by 3 to scale values to beats per minute. A beat was defined as the beginning of the contractile movement of the organoid. A beat did not need reach conclusion to be counted. If multiple beating regions were observed then the beating of the largest multi-cell structure was used to calculate the beating rate.

Statistics
The data are generally presented as the means of number of replicates ± SD. All data were graphed and analyzed for significance using a Student's T-test. For ATP activity assays p-values were considered significant when <0.01. For beating kinetic assays p-values were considered significant when <0.05. Data samples were eliminated from the experimental groups if they fell outside of two SDs from the experimental group averages. Sample sizes (generally n = 5 or n = 6, depending on the experiment as described) were determined based on preliminary experiments. These sample sizes, with the typically observed SDs, allowed statistical significance at $\alpha = 0.05$ with statistical power greater than 80 %. The IC_{50} was calculated using the Graph Pad Prism© software.

II. RESULTS AND DISCUSSION

Organoid production and viability
Liver organoids were successfully formed after 4 days whereas it took 7 days for the cardiac organoids to allow for self-propagating beating to occur. Viability for both was confirmed to be greater than 95 % using live/dead imaging.

Organoid viability following exposure
Live/dead staining of liver organoids was used to visualize indications of cytotoxicity due to exposure to glyphosate. Integrity and viability of liver organoids began to show a steady reduction when exposed to glyphosate concentrations from 250 µM to 2.5 mM. Cardiac organoid responses tended to occur at higher doses than displayed for liver and were more in line with IC50's revealed by ATP testing, described below. The cardiac organoids maintained integrity during all testing, but cell death occurred when exposed to glyphosate concentrations from 2.5 to 25 mM.

Organoid ATP activity
After testing original three doses (n ≥6) centred on doses described in the literature, two further trials narrowed the range containing the IC_{50}. The IC_{50} values were found to be similar for cardiac and liver organoids for glyphosate, i.e. 10.85 and 10.53 mM, respectively. For liver organoids, 10 mM of glyphosate resulted in a statistically significant decrease in ATP activity (p <0.001) and for cardiac organoids, 5 mM of glyphosate resulted in statistically significant decrease in ATP activity (p <0.001).

Cardiac organoid beating rates
The time points to test physiological reactions to glyphosate exposure were chosen based on previous studies testing calcium channel impairment in 2D cardiomyocyte populations and defined as immediate (30 minutes), short term (1 day), and long term (2 days). Although not statistically significant due to high standard deviations among the samples, glyphosate demonstrated toxicity at 250 µM and higher, with beat rates at 250 µM slowing on day one among all organoids and two of the three organoids ceasing beating on day 2. At 2.5 mM, exposure for one day caused two spheroids to cease beating entirely, with one-third showing a 50 % reduction in beat rate.

By day 2 all organoids ceased beating.

Study conclusion
Glyphosate live/dead imaging varied greatly, with significant cell death in liver organoids and little cell death in cardiac organoids at 2.5 mM. ATP values recorded at 10 mM showed a large difference between liver and cardiac organoids (0.568 and 0.804, respectively), displaying a more gradual decrease in cell viability for the liver organoids as compared to the cardiac organoids. The IC_{50} values of liver and cardiac organoids were found to be similar i.e. 10.53 and 10.85 mM, respectively. When cardiac organoids were exposed to glyphosate at concentrations from 0.25 mM on an effect on beating rate was observed with a complete stop of beating at 2.5 mM after 2 days of exposure.

Assessment and conclusion by applicant:
In this study the toxicity of glyphosate for liver and cardiac organoids was investigated in the concentration range from 25 µM to 25 mM. The endpoints considered were cell viability, ATP activity and beating rate of the cardiomyocytes. Glyphosate was shown to reduce organoid integrity and viability at doses from 250 µM to 2.5 mM. The IC_{50} values based on ATP activity of liver and cardiac organoids were found to be 10.53 and 10.85 mM, respectively. When cardiac organoids were exposed to glyphosate at 0.25 mM a non-statistically significant effect was found on beating rate. Exposure to 2.5 mM for 2 days resulted in all organoids stopping beating.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, no positive controls were used to validate the organoid test systems and the concentrations at which most of the effects have been observed are physiologically not feasible in *in vivo* experimental models.

Reliability criteria for *in vitro* toxicology studies according to the applicant

Publication: Forsythe *et al*., 2018	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity and source were not reported.
Only glyphosate acid or one of its salts is the tested substance	N	Also lead, mercury and thallium were assessed.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y?	Liver and cardiac organoids. Origin of hepatic cells not sufficiently documented.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Partly	Test concentrations at which effects were seen were > mM.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive controls used.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		

Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, no positive controls were used to validate the organoid test systems and the concentrations at which most of the effects have been observed are physiologically not feasible in *in vivo* experimental models.		

Assessment and conclusion by RMS:
It is agreed with the applicant's summary of the study, but not with its conclusion. In contrast to the applicant, the study is not considered relevant for risk assessment. From a scientific point of view, the study may be interesting, however, its regulatory value is limited. In addition, the deviations/shortcomings noted above by the applicant invalidate the study.

B.6.8.2.16. Public literature – Mechanism underlying effect of long-term exposure of pesticides on DNA integrity (study for which the RMS requested a summary in order to further justify the categorization)

Information on the study

Data point:	CA 5.8.2
Report author	Alleva R. *et al.*
Report year	2018
Report title	Mechanism underlying the effect of long-term exposure of pesticides on DNA integrity
Document source	Environmental Toxicology (2018), Vol. 33, No. 4, pp. 476-487
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

Pesticides, including herbicides, insecticides and fungicides, are widely used in intensive agriculture. Recently, the long-term effects of pesticide exposure were found to be associated with many diseases. In this study, the long-term effect of low-level exposure to a mixture of pesticides including glyphosate (batch and purity not reported) on DNA damage response (DDR) in relation to individual detoxifying variability. A residential population chronically exposed to different pesticides, among them the herbicide glyphosate, was enrolled. Serum samples were collected from the resident population and serum paroxonase 1 (PON-1) activity and 192 Q/R polymorphism and DDR were evaluated at three different periods of pesticide exposure: no exposure, when the pesticides were not used (February-March); low exposure, when pesticides were used occasionally (October-November); and high exposure, in which pesticides were used regularly on a weekly basis (May-June). In parallel, the pesticide content in the environment was evaluated.

In residents chronically exposed to pesticides, accumulation of DNA lesions was observed. OGG1-dependent DNA repair activity was decreased in relation to pesticide exposure. The increase of DNA lesions and pesticide levels in the intensive pesticide-spraying period was independent on PON-1 activity. However, as the environmental monitoring did not identify glyphosate in residential dust and consequently the human biomonitoring data have not been considered further in this summary.

In a second step, human bronchial epithelial (BEAS-2B) and neuronal cells (SY5Y-5Y) were used as a model for *in vitro* evaluation of the mechanistic effect of pesticides. The cells were selected because pesticides are predominantly absorbed through airways and the neurons are the targets for these compounds. Glyphosate-induced cytotoxicity was assessed for a concentration range of 10-1000 µM using propidium iodide (PI) staining. Glyphosate was investigated *in vitro* for induction of intracellular reactive oxygen species (ROS) at non-cytotoxic concentrations of 10 and 100 µM, followed by evaluation of DNA damage and DNA repair.

In BEAS-2B and SY5Y-5Y cells, the pesticides induced mitochondrial dysfunction leading to ROS formation. ROS from mitochondria induced DNA damage, which in turn induced OGG1-dependent DNA repair activity through 8-oxoguanine DNA glycosylase 1 (OGG1) expression and activation. Even though OGG1 was overexpressed, an inhibition of its activity, associated with DNA lesion accumulation, was found at prolonged pesticide-exposure. A post-translational regulation of OGG1 by pesticide may be postulated. Taken together, long-term exposure to low levels of pesticides affects DDR resulting in accumulation of DNA lesions that eventually may lead to cancer or neurological disorders.

Materials and methods

Test Material:	Glyphosate[8]
Origin:	Sigma
Lot/Batch number:	Not reported
Purity:	Not reported
CAS#:	Not reported
Vehicle:	DMEM culture medium
Positive control:	Not employed in this study

Cell culture and treatments
BEAS-2B (ATCC CRL9609), and SHSY-5Y cells (ATCC CRL2266 were grown in DMEM medium with antibiotics and 10% FBS, and regularly checked for absence of mycoplasma contamination using the PCR Mycoplasma Test. The cells were exposed to glyphosate concentrations of 10-1000 µM. For chronic exposure, both cell types were exposed to 100 µM glyphosate over a period of 6 months with a medium change three times per week. The superoxide scavenger Tiron and the general ROS scavenger NAC were added at 10 mM 2 hours before treatment.

Cell proliferation and cell death assay
Cells were seeded at 10^4 cells/well in a 96-well plate, allowed to attach overnight, and treated with glyphosate concentrations of 10, 50, 100, 500, and 1000 µM. After 24–48–72 h incubation, 50 µL of crystal violet (2% crystal violet in 2% ethanol) were added, and incubated for 5 minutes. After four washes, 200 µL of isopropanol was added to dissolve the crystals, and absorbance read at 570 nm in an ELISA plate reader (Sunrise, Tecan, Männedorf, Swiss), and the results expressed as rate of proliferation (abs/time). Apoptosis was detected by annexin V/propidium iodide double staining. Briefly, cells were seeded in six-well plates by density of 2×10^5 cells/well, allowed to attach overnight, and treated with or without glyphosate (10 µM and 100 µM) for 24 h. After incubation, the cells were collected, resuspended in 100 µL of binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, and 2.5 mM $CaCl_2$) and incubated with 1 µL of FITC-conjugated Annexin-V and 10 µL of propidium iodide (PI) for 15 minutes at room temperature in the dark. Annexin V-FITC and PI fluorescence was monitored by flow cytometry (FACS Calibur, Becton Dickinson). Cells that stained positive for Annexin V were classified as early apoptotic cells; cells positive for both PI and Annexin V staining were classified as late apoptotic cells; PI positive and Annexin V negative were classified as necrotic cells; PI and Annexin V negative cells were classified as live cells.

Assessment of ROS
Intracellular reactive oxygen species (ROS) levels were estimated using the fluorescent dye 2'7'-dichlorofluorescein diacetate (DCFDA; oxidized by hydrogen peroxide to DCF). BEAS-2B and SHSY-5Y cells (2×10^5) were seeded in six-well plates, supplemented with 20 µM DCFDA per well, and treated with 10 and 100 µM glyphosate. After treatment, the florescent probe was removed, the cells washed and re-suspended in PBS, and analysed by flow cytometry (FACS Calibur, Becton Dickinson). The level of ROS was expressed as fold increase in fluorescence with respect to control (untreated cells), or as mean fluorescence intensity (MFI).

DNA damage
DNA breaks and oxidized purine and pyrimidine bases were measured using the Comet assay. Briefly, glyphosate-treated and control cells were embedded in agarose on a microscope slide, lysed with Triton X-100

[8] No information on the test material / glyphosate the resident people were chronically exposed to. Information on glyphosate provided in this summary refers to the test material used in the in vitro studies.

and 2.5 M NaCl to produce nucleoids, and treated with 0.3 M NaOH/1.0 mM EDTA before electrophoresis in this solution. Oxidized bases were detected by including an extra step, in which nucleoids in the gel are digested with a repair endonuclease specific for oxidized pyrimidines (endonuclease III, ENDO III) or recognizing altered purines, including 8-oxoGua (8-oxoGuanine, formamido pyrimidine glycosylase, FPG). Slides were incubated for 30 minutes with 50 µL of either buffer, FPG, or ENDO III (generously given by Prof. Andrew Collins, University of Oslo, Oslo, Norway). DNA strand breaks (SBs), with or without enzymatic treatment, were estimated as arbitrary units (au). Oxidized purine and pyrimidine bases were calculated by subtracting the value without enzyme incubation (ie, SBs) from the value with enzyme incubation. The extent of DNA migration was evaluated by visual scoring by an independent observer.

DNA repair assay
The activity of OGG1-dependent DNA repair was evaluated as capacity of cell extract to repair the oxidized purine (8-oxoGua) introducing DNA breaks detected as previously described. Briefly, cell extract was prepared from 2.0 x 10^6 cells in extract buffer (45 mM Hepes, 0.4 M KCl, 1.0 mM EDTA, 1 mM DTT, and 10% (v/v) glycerol adjusted to pH 7.8 with KOH), and was cryopreserved at -80°C until used in the DNA repair assay. Lysate (50 µL) was supplemented with 12 µL of extract buffer containing 1% Triton X-100 and then centrifuged for 5 min at 4°C at 1400g. The supernatant was diluted with four volumes of reaction buffer with 0.25 mM EDTA, 2% glycerol, and 0.3 mg/mL BSA, pH 7.8. The protein extract (50 µg) was added over a slide with a substrate consisting of nucleoid DNA with oxidized purine bases from A549 cells previously exposed to Ro 19–8022 plus light, which specifically induces 8-oxoGua formation. The slides were incubated for 45 min at 37°C and comet assay (alkaline unwinding, electrophoresis, neutralization, staining, and evaluation) was carried out as described above. The time-course of break production on the specifically damaged DNA substrate is a measure of repair capability.

Western blot analysis
3 x 10^5 cells per well in six-well plates were lysed in the RIPA buffer containing Na_3VO_4 (1 mM) and protease inhibitors (1 mg/mL). The cell lysate protein (50 µg per lane) was resolved using 4%-12% SDS-PAGE (Life Technologies), transferred to nitrocellulose membranes and incubated overnight with anti-OGG1 (Origene, Rockville, MD). Actin (Bethyl, Montgomery, TX) was used as loading control. After incubation with an HRP-conjugated secondary IgG (Sigma), the blots were developed using the ECL detection system (Pierce Biotechnology, Rockford, IL). Band intensities were visualized by ChemiDoc using the Quantity One software (BioRad, Hercules, CA).

Statistical analysis
Results were expressed as mean ± SD, or median (25^{th} percentile-75^{th} percentile). Comparisons among groups of data were made using one-way analysis of variance (ANOVA) with Tukey post hoc analysis. The two-tailed Student's t-test was used to compare two groups. The data were analysed by the Statistical Package Social Sciences (version 19) software (SPSS, Chicago, IL) and differences with a P value of <0.05 that are considered significant.

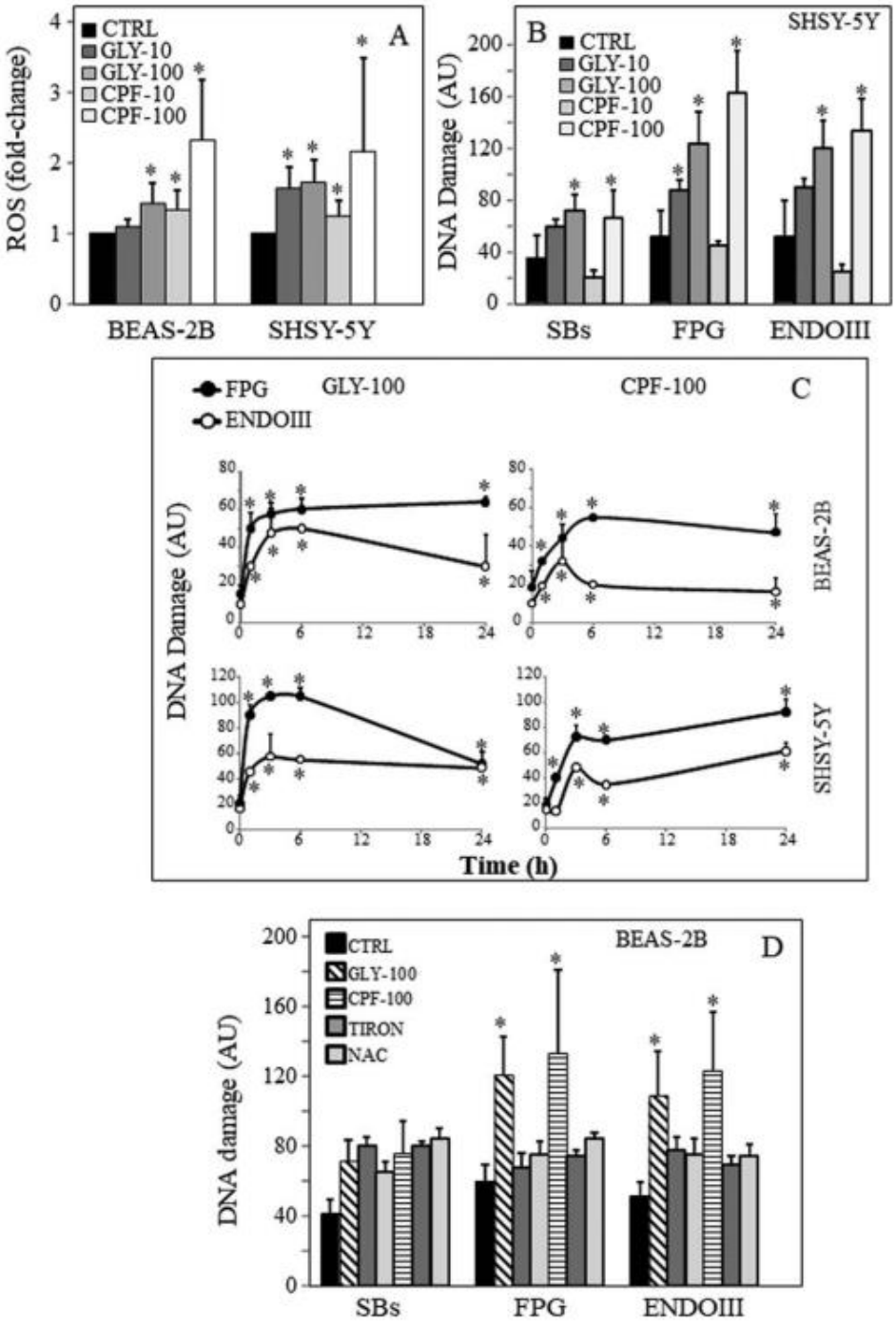
Results

Environmental monitoring did not identify glyphosate in residential dust and consequently, the human biomonitoring data have not been considered further in this summary.

Pesticides induce DNA damage by mitochondrial ROS formation
Bronchial epithelial cells (BEAS-2B) and neuronal cell line (SHSY-5Y) were used as models to evaluate *in vitro* pesticide-induced DNA damage response (DDR). Glyphosate exposure was shown to affect cell proliferation without inducing cell death. Glyphosate induced formation of reactive oxygen species (ROS) in both cell lines (Fig. 1A), with consequent DNA damage evaluated as SBs and oxidized DNA bases (FPG- and ENDOIII-sites) (Fig. 1B). Accumulation of FPG and ENDOIII lesions was observed after 3 hours of pesticide incubation (Fig. 1C).

To determine a direct relationship between intracellular ROS levels and pesticides-induced DNA damage through mitochondrial destabilization, the cells were pre-treated with the superoxide scavenger Tiron (10 mM), ROS scavenger N-acetyl cysteine (NAC, 10 mM), and DNA damage was induced by glyphosate treatments (100 µM) for 24 h. The inhibition of ROS by Tiron and NAC paralleled the reduction of pesticide-induced DNA damage (Fig. 1D). The implication is that pesticides affect mitochondrial homeostasis by inducing ROS production (mtROS), which in turn induce DNA damage.

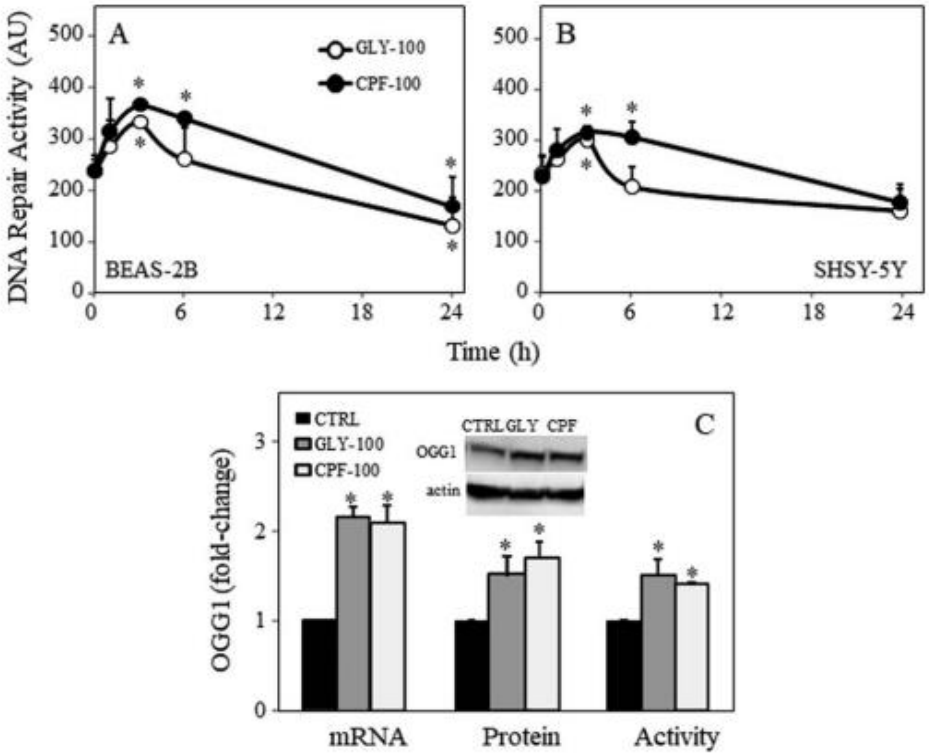
Figure 1. Pesticide exposure induces reactive oxygen species (ROS)-mediated DNA damage. Human bronchial epithelial cells (BEAS-2B) and neuronal SHSY-5Y cell lines were treated with glyphosate (GLY) at 10 µM and 100 µM and ROS formation (A) and DNA damage evaluated (B). Kinetics of oxidized DNA bases (FPG and ENDOIII) formation induced in BEAS-2B and SHSY-5Y cell lines at 100 µM glyphosate concentration (C). BEAS-2B cells were treated with 100 µM glyphosate and DNA damage was evaluated in the presence or absence of Tiron (10 mM) and NAC (10 mM) (D). The results are expressed as fold change with respect to untreated cells (control), or as arbitrary units (AU) of three experiments. Comparisons among groups were determined by one-way ANOVA with Tukey post hoc analysis. *Significant differences with untreated cells, with $P < 0.05$.



Pesticides-induced DNA repair inhibition leads to DNA lesion accumulation

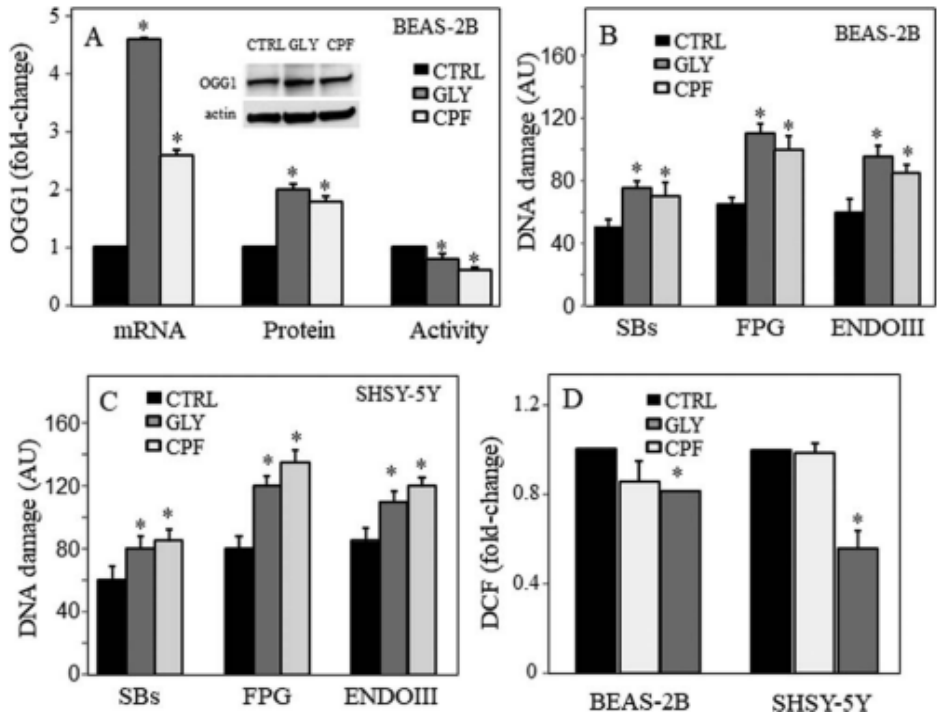
As a consequence of DNA damage, sensors that induce DDR are activated. According to the type of DNA lesions, cells use distinct mechanisms of DNA repair. Among them, base excision repair (BER) is the major pathway used for oxidative damage repair, where 8-oxoguanine DNA glycosylase 1 (OGG1) plays an essential role in the removal of 8-oxo-guanine (8-OHdG, FPG-sites) from nuclei. Glyphosate induced OGG1-dependent DNA repair activity within 1-6 hours of exposure, while reducing its activity at prolonged time of exposure in both cell lines (Fig. 2A, B).

Figure 2. Effect of glyphosate on DNA repair activity in human bronchial epithelial cells (BEAS-2B) and neuronal SHSY-5Y cells. DNA repair activity was evaluated over time (A, B). BEAS-2B cells were treated at 100 µM for 3 hours and OGG1 mRNA, protein measured by western blot (insert), and activity evaluated in cellular extracts (C). The results are expressed as fold change with respect to untreated cells (control), or as arbitrary units (AU) of three experiments. Comparisons among groups were determined by one-way ANOVA with Tukey post hoc analysis. *Significant differences with untreated cells, with P <0.05.



As shown in Figure 2C the pesticide-induced DNA repair activity was associated with increased gene and protein expression of OGG1. Conversely, the pesticide-induced OGG1 gene and protein expression was not associated with an increase in its activity in BEAS-2B cells chronically exposed to glyphosate (Fig. 3A). The reduction in OGG1-dependent DNA repair activity paralleled DNA lesion accumulation in both cell lines (Fig. 3B, C), and was independent of ROS formation (Fig. 3D).

Figure 3. DNA damage response after chronic (6-month) exposure to 100 µM glyphosate. OGG1 expression (mRNA and western blot) and activity (A), DNA damage (B, C), and ROS (D) formation were evaluated in BEAS-2B and SHSY-5Y cells. The results are expressed as fold change with respect to untreated cells (control), or as arbitrary units (AU) of three experiments. Comparisons among groups were determined by one-way ANOVA with Tukey post hoc analysis. *Significant differences with untreated cells, with P <0.05.



Conclusion

Based on the experimental findings of this publication, glyphosate induces DNA damage by mitochondrial ROS formation in BEAS-2B and SHSY-5Y cells. As a consequence of DNA damage, precisely OGG1-dependent base excision repair is activated. The data support a role for DNA repair mechanisms and suggest that chronic exposure to pesticides affects the DNA damage response.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: Purity and source not reported. No positive control. Only one or two concentrations of glyphosate were tested. Comparisons are to untreated cells rather than negative controls. This publication is considered relevant for the risk assessment of glyphosate (5.4.1 case B) but the reliability of the study is unassignable/unreliable. Therefore the study is considered as supplementary information.

Further points for clarification:
In this paper, the authors undertook a range of biomonitoring assessments in a population of human volunteers with long-term but low-level exposure to a mixture of pesticides. Environmental monitoring did not identify glyphosate in residential dust and consequently, the human biomonitoring data have not been considered further in this summary.

In a number of *in vitro* experiments, pesticide-induced DNA damage was investigated. Two different cell lines were treated with glyphosate and chlorpyrifos ethyl; only the glyphosate findings have been considered in this assessment. Glyphosate was shown to induce DNA damage by mitochondrial ROS formation and subsequent activation of DNA damage response mechanisms. It was shown that chronic exposure to pesticides affects the DNA damage response.

In general, the methodology is adequately described. However, there are significant details missing with respect to the DNA damage and DNA repair assays that mean the reliability of the reported results is uncertain. The general comet assay procedures are poorly described, and no information is provided regarding cell selection for scoring, identification of cloud/ghost/hedgehog cells or categorization of DNA migration. Other significant flaws are the lack of clear detail regarding treatment exposure time in each experiment and the use of untreated cells for comparison of effects. There is no indication that these control cells were treated in the same way (i.e. under the same culture conditions, for the same exposure period and with the same

addition of ROS scavengers). ROS data are provided solely as fold-change rather than actual values obtained; thus background variation cannot be determined. A lack of historical negative and positive control data also prohibits the assessment of intra- and inter-experiment variability. Further, no positive control was included in the *in vitro* experiments and only one or two concentrations of glyphosate were investigated. There is no information on the test material batch and purity.

Assessment and conclusion by RMS:

In this study the environmental presence of pesticides in dust and air samples was evaluated against the activity of paraoxonase-1 (PON-1) in serum samples of residents living in an area of apple orchards in Trento, Italy during three periods of the growing season. As glyphosate was not among the residues of pesticides observed in the environmental samples, it is agreed that the results were not further discussed in the summary.

Additionally, bronchial epithelial cells (BEAS-2B) and neuronal cell line (SHSY-5Y) were used as models to evaluate *in vitro* pesticide-induced DNA damage response (DDR) by exposing them to pure glyphosate and chlorpyrifos ethyl. The latter is not discussed further in the summary. Under the study conditions glyphosate induces DNA damage by mitochondrial ROS formation in BEAS-2B and SHSY-5Y cells. Increased OGG1-dependent DNA repair activity, associated with gene and protein upregulation of the DNA glycosylase OGG1, was found in cells after 3 h of pesticide treatment, then declining at prolonged time of incubation.

It is agreed with the methodological shortcomings described by the applicant. Therefore, the study is considered as supplementary data only.

B.6.8.2.17. Public literature – Effects on ovarian function of pregnant mice, secretion hormones and sex ratio (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.8.2
Report author	Ren X. et al.
Report year	2018
Report title	Effects of glyphosate on the ovarian function of pregnant mice, the secretion of hormones and the sex ratio of their fetuses
Document source	Environmental Pollution (2018), Vol. 243, Pt B, pp. 833-841
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

Glyphosate is the active ingredient of the commercial formulation Roundup, which is used worldwide. This study aimed to investigate the toxic effects of pure glyphosate or Roundup on pregnant mice and their foetuses during pregnancy. From gestation days (GDs) 1-19, ICR mice were orally administered distilled water, 0.5% glyphosate solution or 0.5%-glyphosate Roundup solution. The ovaries and serum were collected at GD19.

Materials and methods

Test Material:	N-(phosphonomethyl)glycine) (I) Roundup (II)
Origin:	Shanghai Ryon Biological Technology Co. Ltd. (Shanghai, China) (I) Sinochem Crop Protection Products Co., Ltd. (Shanghai, China) (II)

Lot/Batch number:	Not reported
Purity:	Not reported
CAS#:	Not reported
Vehicle:	Distilled water
Test Animals:	
Species	Mouse
Strain	ICR
Age/weight on arrival	10 weeks/ weight not reported
Source	Nanjing Qinglongshan Experimental Animal Center (Nanjing, China)
Housing	Individually
Acclimatisation period	1 week
Diet	Not specified
Water	*Ad libitum*
Environmental conditions	
Temperature	23 ± 2 °C
Light/dark cycle	12 hour light/dark cycle
Humidity	50 ± 10 %

Preparation of chemicals
Glyphosate and Roundup were dissolved in distilled water to obtain a 0.5%-active ingredient solution (w/v, 5 g glyphosate/1 L solution). Then, 0.5% glyphosate and Roundup solution were orally administered through the daily drinking water (pH was controlled at 7.2 ± 2).

Animal treatment and sampling
After one week of acclimatisation, eleven-week-old female and male ICR mice were cohoused from 5.00 p.m. to 8.00 a m. daily at a ratio of 1:2 until they mated. A vaginal smear was conducted the following morning to confirm coitus and pregnant females were separated into individual cages, this day was identified as day 1 of gestation. A total of 15 pregnant mice were randomly allocated into three treatment groups: CON (control group, n = 5), GLP (0.5% glyphosate-treated group, n = 5), and RU (0.5% Roundup®- treated group, n = 5). Animals were administered the test substance daily through drinking water from gestation day (GD) 1 to GD 19 and sacrificed on the last day of dosing. Weekly body weight gain, daily feed intake and water consumption were recorded. Foetuses were obtained by hysterectomy. The weight and number of litters, and individual anogenital distances, the length of the body and tail and sex ratios were recorded and calculated. All organs' wet weights were measured.

Histological preparation
Ovarian tissue was fixed in 4% paraformaldehyde solution for 24 h and then dehydrated with alcohol, clarified with xylene and finally embedded in paraffin, which was sectioned into 5 mm slices for haematoxylin-eosin staining (H&E). At least 3 paraffin blocks of each group were chosen, and each of them was sectioned to get 6 consecutive slices to count follicle numbers. The primary follicle, secondary follicle, mature follicle, atretic follicle and metoarion in each slice were identified and counted for statistical analysis.

Serum hormone concentration assay
The blood of dams was collected and placed at room temperature for 2 h before being centrifuged (3500 rpm, 15 min, 4°C) to get the serum. To explore the effect of glyphosate exposure on endocrine disruption, serum progesterone and oestrogen concentration were assayed with the ELISA reagent kit.

Analysis of gene expression
The following tissues were stored at -80 °C for reverse transcription-polymerase chain reaction (RTPCR): Hypothalamus, pituitary and ovary. Total RNA was extracted from the tissue with the ISOGEN 2 reagent kit (NIPPON GENE CO., LTD., Japan) according to the manufacturer's protocols. RNA concentration was determined by a spectrophotometer and its purity was evaluated by the value of A260/A280 using Nanodrop 8000. Then, RNA was reverse transcribed to cDNA with PrimeScript RT Master Mix (TAKARA BIO INC.). The obtained cDNA was used as a template for the SYBR Premix Ex Taq (TAKARA BIO INC.) PCR kit, which was used for real-time PCR. The expressions of GnRH (gonadotropin-releasing hormone), LH (luteinizing hormone), FSH (follicle-stimulating hormone), LHR (luteinizing hormone receptor), FSHR (follicle-stimulating hormone receptor), StAR (steroidogenic acute regulatory protein), Cyp11a1 (cytochrome P450, family 11),

3b-HSD (3beta-hydroxysteroid dehydrogenase), Cyp17a1 (cytochrome P450, family 17), Cyp19a1 (cytochrome P450, family 19), and cAMP (cathelicidin antimicrobial peptide) genes were determined. The relative gene expressions were normalized to b-actin expression. The primers were designed and provided by GenScript Bio-Tech Co., Ltd. (Nanjing, China).

Antioxidant enzyme assay

To determine the oxidative status of the ovary, ovary homogenate was centrifuged to obtain the supernatant (3500 rpm, 15 min, 4°C). Malondialdehyde (MDA) content, catalase (CAT), total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activity and total antioxidant capacity (T-AOC) in the ovary and serum were assayed by a commercial reagent kit purchased from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China).

Statistics

Data were analysed with SPSS Statistics 20.0 software and GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Statistics were presented as the mean ± standard error of the mean (SEM) and analysed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Statistical significance was set as $p < 0.05$.

Results

Maternal and foetal variables

From GD1-19, a significant decrease in both daily water consumption and body weight gain was detected in GLP and RU compared to CON ($p < 0.01$, Table 1). Additionally, the body weight of CON on GD19 was significantly higher ($p < 0.01$) than that of GLP and RU. Meanwhile, there was no obvious change in feed intake among the three groups during the 19 days (Fig. 1). The results suggested that GLP and RU could considerably affect the physical development of pregnant mice or their foetuses during pregnancy. There was a reduction in foetal weight and number in GLP and RU (Table 1), while statistically significant differences were not observed in anogenital distance, body length and tail length among the three groups (Table 2). Simultaneously, the ratio of females to males decreased in GLP-treated foetuses ($p < 0.05$) (Table 1). With respect to organ weight, the ovary weight of RU was significantly lower ($p < 0.05$) than that of CON but similar to that of GLP. The liver weight of GLP-treated mice significantly decreased compared with CON and RU ($p < 0.05$) (Table 3).

Figure 1. Effect of glyphosate exposure on feed intake and body weight in pregnant mice during GD1-19 (mean ± SEM). Different capital letters indicate statistically significant differences, $p < 0.01$.

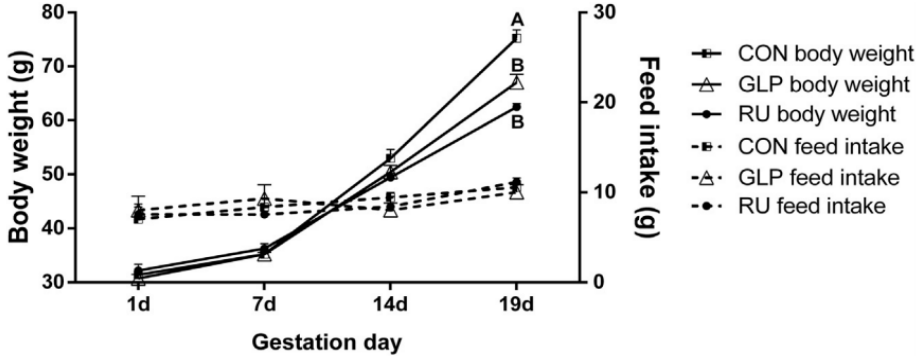


Table 1. Effects of glyphosate exposure during pregnancy on the development and reproductive performance of pregnant mice

Items	CON	GLP	RU	*P*
Daily water consumption (ml/d)	11.02 ± 0.64^{A}	7.32 ± 0.16^{B}	7.79 ± 0.14^{B}	0.001
Daily feed intake (g/d)	8.72 ± 0.27	8.43 ± 0.20	8.55 ± 0.26	0.728
Body weight gain (g)	43.83 ± 0.56^{A}	36.30 ± 1.01^{B}	30.27 ± 1.57^{B}	0.000
Fetal weight (g)	21.44 ± 0.62	20.24 ± 1.29	17.36 ± 1.75	0.131
Number of fetuses (n)	15.20 ± 0.58	14.80 ± 1.24	13.80 ± 0.66	0.529
Average fetal weight (g)	1.48 ± 0.09	1.48 ± 0.04	1.39 ± 0.13	0.722
Females to males ratio of fetuses	1.77 ± 0.25^{a}	0.76 ± 0.10^{b}	1.62 ± 0.31^{ab}	0.022

Each value represents the mean ± SEM of the group (n = 5).
Different letters indicate statistically significant differences. a, b $p < 0.05$ and A,B $p < 0.01$.

Table 2. Effects of glyphosate exposure during pregnancy on foetal development

Items	Female				Male			
	CON	GLP	RU	*P*	CON	GLP	RU	*P*
Number (n)	8.25 + 1.18	7.33 + 0.67	7.33 + 1.45	0.808	5.75 + 1.25	8.33 + 0.88	5.67 + 1.20	0.273
Anogenital distance (mm)	2.47 ± 0.17	2.32 ± 0.18	2.39 ± 0.06	0.763	3.44 ± 0.29	3.21 ± 0.24	3.42 ± 0.12	0.733
Body length (mm)	44.25 ± 0.61	45.23 ± 2.03	44.36 ± 0.63	0.866	43.92 ± 0.41	45.52 ± 2.44	44.96 ± 0.71	0.801
Tail length (mm)	12.20 ± 0.32	11.54 ± 0.39	12.56 ± 0.32	0.160	12.07 ± 0.18	11.61 ± 0.41	12.56 ± 0.47	0.257

Each value represents the mean ± SEM of the group.

Table 3. Effects of glyphosate exposure during pregnancy on organ weight (g) in pregnant mice

Organ	CON	GLP	RU	*P*
Uterus	3.95 ± 0.51	4.26 ± 0.68	3.73 ± 0.84	0.865
Placenta	2.65 ± 0.41	2.05 ± 0.12	2.01 ± 0.31	0.294
Ovary	0.13 ± 0.04^{a}	0.09 ± 0.03^{ab}	0.08 ± 0.02^{b}	0.033
Liver	3.32 ± 0.14^{a}	2.39 ± 0.19^{b}	3.28 ± 0.22^{a}	0.015
Kidney	0.50 ± 0.01	0.49 ± 0.02	0.52 ± 0.05	0.779
Spleen	0.24 ± 0.06	0.35 ± 0.21	0.33 ± 0.08	0.863
Heart	0.20 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.456
Lung	0.29 ± 0.03	0.24 ± 0.01	0.30 ± 0.03	0.137

Each value represents the mean ± SEM of the group (n = 5).
Different small letters indicate statistically significant differences, $p < 0.05$.

Ovarian histological observation

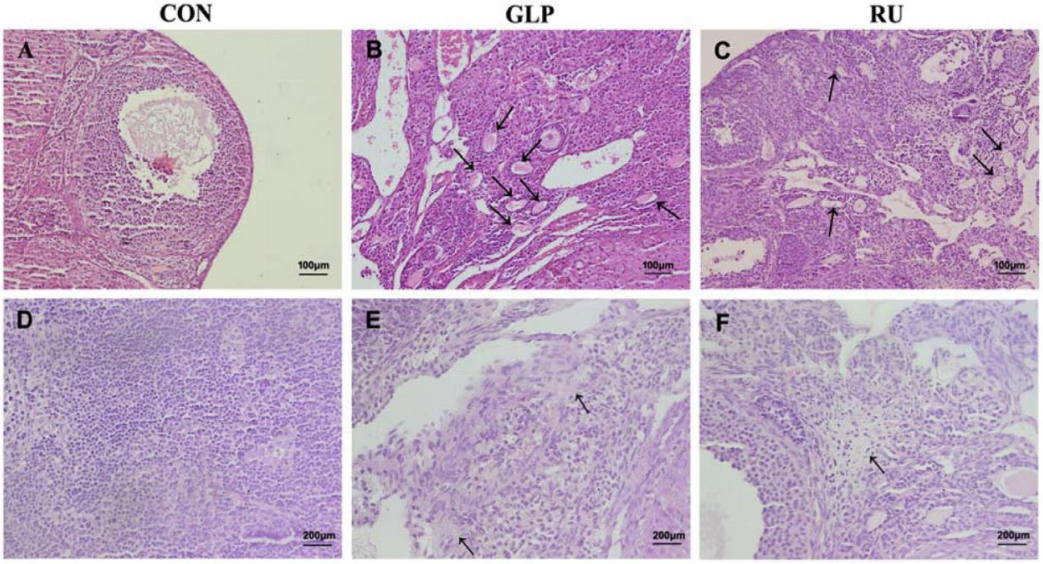
A significant increase in the number of atretic follicles ($p < 0.05$) (Table 4) was observed in GLP (Fig. 2B) compared to CON (Fig. 2A). The number of mature follicles in GLP was significantly lower than that in CON ($p < 0.05$) (Table 4). Moreover, an excessive proliferation of ovarian fibroblasts and deposition of extracellular matrix (Fig. 2E and F) was noticed in GLP and RU.

Table 4. Effects of glyphosate exposure during pregnancy on types of ovarian follicles and metoarion in pregnant mice

Items	CON	GLP	RU	*P*
Primary follicle	6.67 ± 1.33	7.33 ± 2.60	5.00 ± 2.52	0.617
Secondary follicle	11.00 ± 2.08	5.67 ± 0.33	4.00 ± 1.00	0.066
Mature follicle	10.67 ± 0.88^{a}	2.33 ± 0.33^{b}	6.00 ± 1.53^{ab}	0.049
Atretic follicle	1.67 ± 0.88^{b}	14.00 ± 1.00^{a}	6.33 ± 1.20^{b}	0.028
Metoarion	2.67 ± 1.33	3.00 ± 0.58	2.67 ± 0.33	0.926

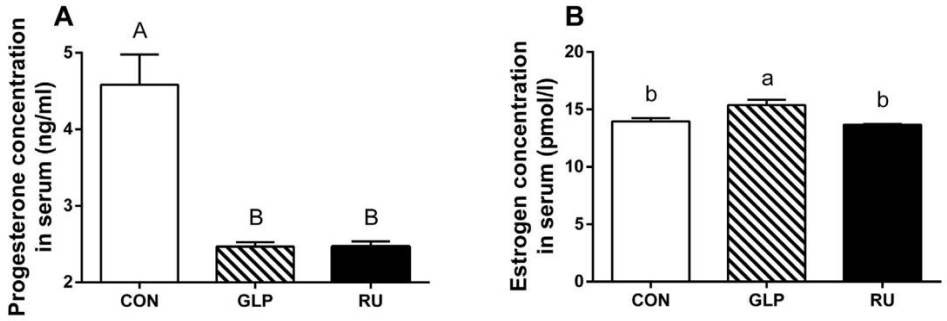
Each value represents the mean ± SEM of the group (n = 4–5).
Different small letters indicate statistically significant differences, $p < 0.05$.

Figure 2. Photomicrographs for ovarian sections of glyphosate-treated mice. (A) shows the normal histological ovarian follicle structure in CON; (B) and (C) show an increase in the number of atretic follicles (black arrows indicate the lesion location) in GLP and RU respectively. (D) shows the normal histological ovarian interstitial cell structure in CON; (E) and (F) show the interstitial fibrosis (black arrows indicate the lesion location) in GLP and RU, respectively. Different small letters indicate statistically significant differences, $p < 0.05$.



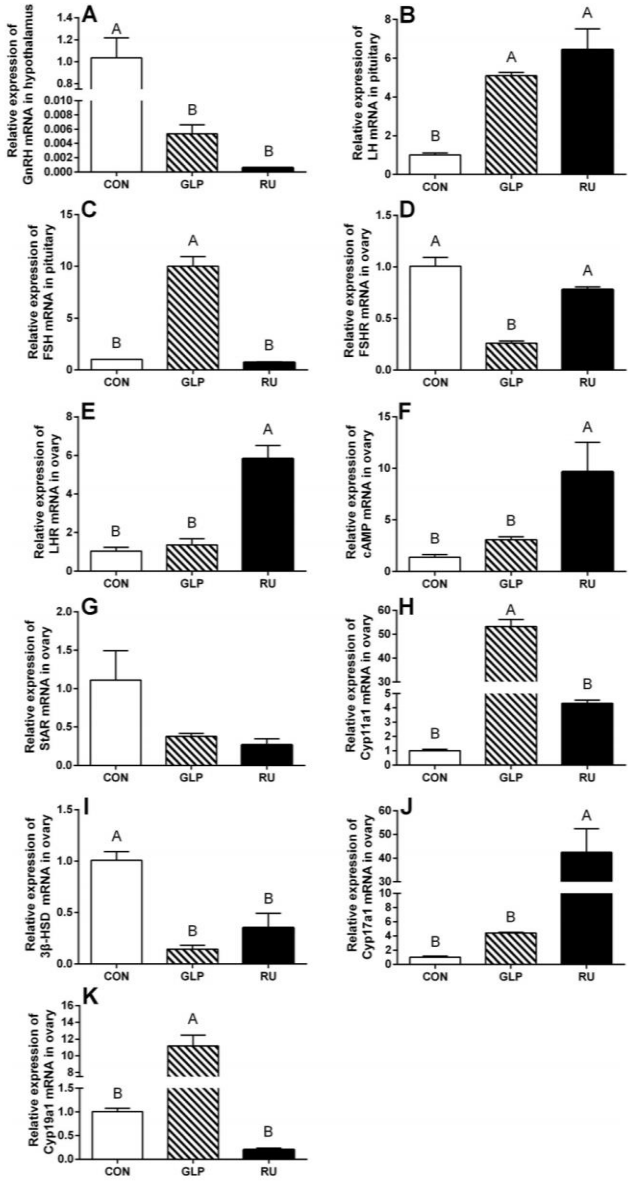
Serum hormone secretion
Compared with CON, serum progesterone concentration in GLP and RU-treated mice dropped significantly ($p < 0.01$) (Fig. 3A). Conversely, elevated serum oestrogen concentration ($p < 0.05$) (Fig. 3B) was determined in GLP compared with CON and RU.

Figure 3. Effect of glyphosate exposure on serum hormone concentration in pregnant mice (mean ± SEM). (A) Progesterone concentration decreased in GLP and RU-treated mice. (B) Estrogen concentration significantly increased in GLP treated-mice. Different letters indicate statistically significant differences. A, b $p < 0.05$ and A,B $p < 0.01$.



Gene expression in the hypothalamic-pituitary-ovarian axis
Hypothalamic GnRH expression decreased in mice exposed to GLP and RU compared with CON, and this difference was statically significant ($p < 0.01$) (Fig. 4A). In pituitary tissue, LH gene expression in GLP and RU significantly increased ($p < 0.01$) compared with CON (Fig. 4B), and the same effect was observed in the expression level of FSH in GLP ($p < 0.01$) (Fig. 4C). With respect to ovarian tissue, LHR expression increased ($p < 0.01$) (Fig. 4E) in RU but the FSHR gene represented a decrease ($p < 0.01$) (Fig. 4D) in GLP after exposure to glyphosate. The cAMP gene, related to PKA activation and the division of oocytes, showed an altered expression level ($p < 0.01$) in RU compared with CON and GLP (Fig. 4F). No significant expression alterations were observed among groups on StAR, the rate-limiting enzyme of progesterone synthesis, whereas a decreased trend could be noticed after exposure in GLP and RU compared with CON (Fig. 4G). Cyp11a1, which catalyses the reactions of pregnenolone production, showed increased expression ($p < 0.01$) in GLP compared with CON (Fig. 4H). 3b-HSD, responsible for the transformation of pregnenolone to progesterone, showed decreased expression in GLP and RU compared with CON ($p < 0.01$) (Fig. 4I). Cyp17a1, the rate-limiting enzyme of oestrogen synthesis, significantly increased ($p < 0.01$) in RU compared with CON and GLP (Fig. 4J). The relative gene expression of Cyp19a1, which catalyses the transformation of androgen to oestrogen, dramatically increased in GLP ($p < 0.01$) (Fig. 4K).

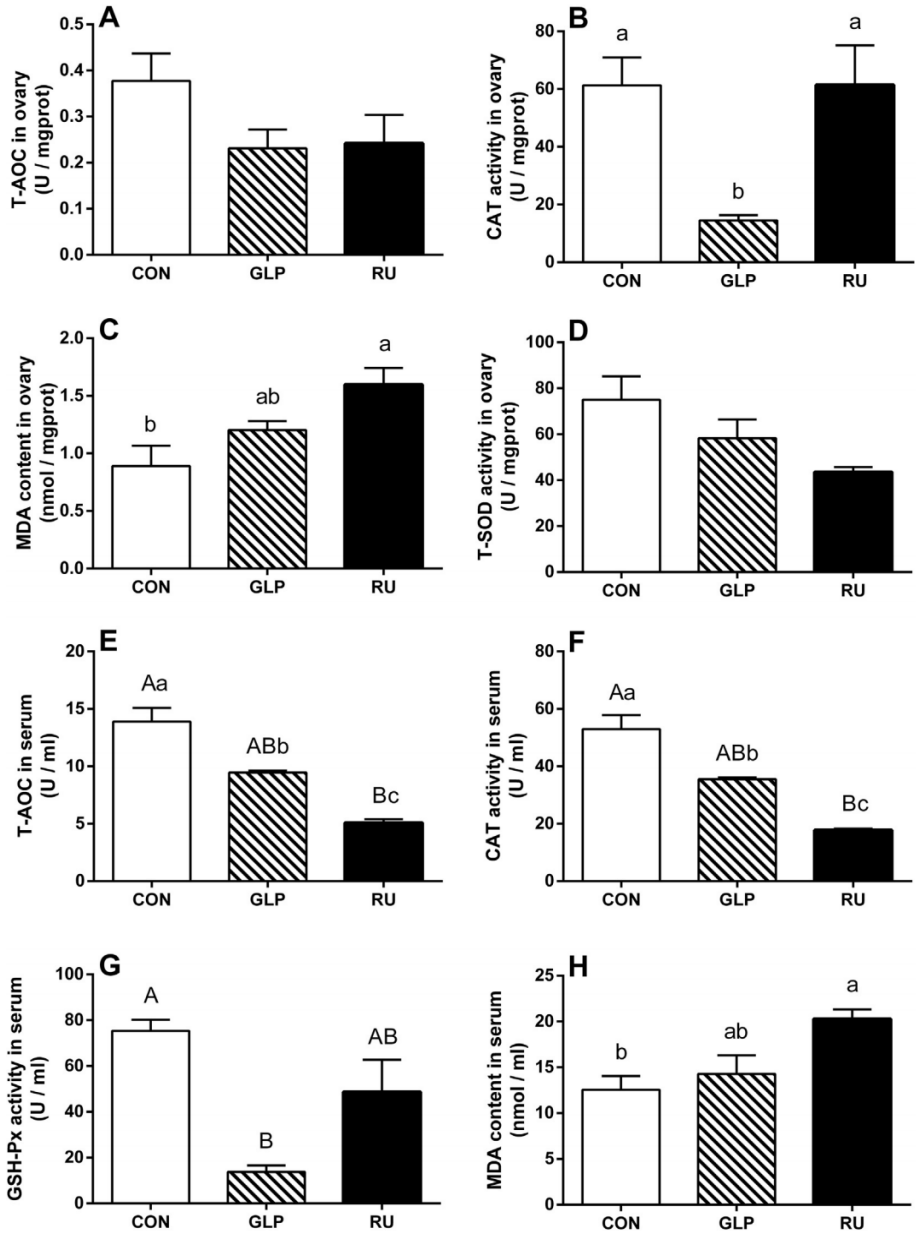
Figure 4. Effect of glyphosate exposure on relative mRNA expression in the hypothalamus, pituitary and ovary of pregnant mice (mean ±SEM). (A) Relative GnRH expression in the hypothalamus. (B) Relative LH expression in pituitary. (C) Relative FSH expression in the pituitary. Relative expression of FSHR (D), LHR (E), cAMP (F), StAR (G), Cyp11a1 (H), 3b-HSD (I), Cyp17a1 (J) and Cyp19a1 (K) in ovary tissue. Different capital letters indicate statistically significant differences, $p<0.01$.



Oxidative status assessment

In GLP, ovarian CAT activity significantly decreased ($p < 0.05$) (Fig. 5B) and MDA content tended to increase (Fig. 5C) compared with CON. There was also a sharp increase ($p < 0.05$) in the MDA content of RU (Fig. 5C). T-AOC (Fig. 5A) and T-SOD (Fig. 5D) activity revealed a decreased trend in GLP and RU. With regard to serum, T-AOC (Fig. 5E), CAT (Fig. 5F) and GSH-Px (Fig. 5G) activity in GLP and RU were markedly lower than in CON ($p < 0.05$ or $p < 0.01$). MDA content (Fig. 5H) in RU significantly increased ($p < 0.05$) compared with CON.

Figure 5. Effect of glyphosate exposure on antioxidant enzyme activity and lipid peroxidation content in the ovary and serum of pregnant mice (mean ±SEM). T-AOC (A), CAT activity (B), MDA content (C), T-SOD activity (D), in ovary tissue. T-AOC (E), CAT activity (F), GSH-Px activity (G) and MDA content (H) in serum. Different letters indicate statistically significant differences. A, b $p < 0.05$ and A,B $p < 0.01$.



Conclusion

The present research showed that glyphosate caused ovarian histopathological alterations, hormonal imbalances and oxidative stress in pregnant mice. Moreover, glyphosate interfered with the expression of steroidogenesis-related genes, resulting in the disruption of progesterone and oestrogen secretion. Prenatal exposure to pure glyphosate could change the sex ratios of foetuses. In addition, the toxic effects of pure glyphosate and Roundup on either ovary function or steroidogenesis are complex. Further studies may help to clarify the underlying mechanisms.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

5.4.1 case b) Relevant but supplementary information: Glyphosate purity not reported. Only one dose level for glyphosate was tested (0.5% solution added to drinking water), it is unclear what actual dose was administered per day. The number of animals used per dose level was too low (5 animals) and only one dose level was considered with no justification for the selection. Insufficient information is given on the biochemical methods used. Glyphosate not sufficiently characterised (purity). Daily dose administered

through drinking water not calculated/provided. This publication is considered unreliable.

The exact composition of the Roundup formulation tested is not stated in the paper (a.i. content, source, surfactant system / co-formulants). It is therefore not possible to confirm whether the product used is the representative glyphosate formulation MON 52276 relevant for the glyphosate EU renewal. Furthermore, in the absence of a concurrent control for each to the component of the formulation, it is not possible to conclude whether the observed effects claimed to be secondary to exposure to glyphosate are due to glyphosate exposure or of one of the other components. The uncertainty associated with whether the product contains polyethoxylated tallow amine (also polyoxyethyleneamine, POEA) or not, suggests that the findings in this paper should be treated with high level of caution.

Assessment and conclusion by RMS:

In this study, glyphosate (GLP) and a unknown Roundup formulation (RU) were administered to pregnant mice during GD 1-19 via drinking water. Body weight gain was decreased in both treated groups compared to the control (-17% GLP, -31% RU). Foetal weight and number were decreased after exposure to glyphosate and roundup formulation; anogenital distance, body length and tail length were not affected. Liver weight was decreased following treatment with glyphosate compared to the control (-28%), no difference was found between the Roundup group and the control group. Ovary weight was decreased in both treated groups compared to control (-31% GLP, -38% RU). Glyphosate exposure resulted in an increase in the number of atretic follicles and a lower number of mature follicles. Treated mice had lower levels of serum progesterone; GLP treatment resulted in increased oestrogen concentration compared to control and RU. Gene expression alterations were found following exposure.

The applicant considers this study to be supplementary data only. The study is carried out with glyphosate and an unknown Roundup formulation. For the results in the Roundup group, side effects caused by co-formulants cannot be excluded. Furthermore, it is unknown if the tested formulation contains polyethoxylated tallow amine (POEA). No justification for the selected dose level or route of administration were given. The route of exposure was via the drinking water, however, details on the actual doses the animals were exposed to are not given in the article. The results indicated systemic toxicity as body weight gain was decreased by 17 and 31% for GLP and RU, respectively and decreased liver weight (-28% or GLP). Therefore, it cannot be determined whether the results described are secondary to maternal general toxicity.

The study is considered unreliable because of the following reasons: the study was not conducted according to any international guideline, no GLP status, the test substances are not sufficiently characterised, unknown dose of exposure following administration via drinking water, small group size (n=5), exposure during GD1-19 without justification for this window of exposure, only one dose tested, individual data missing, no historical control data or positive control.

B.6.8.2.18. Public literature – Glyphosate based herbicide exposure affect gut microbiota, anxiety and depression-like behaviour in mice (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.3
Report author	Aitbali Y. *et al*.
Report year	2018
Report title	Glyphosate based- herbicide exposure affect gut microbiota, anxiety and depression-like behaviors in mice
Document source	Neurotoxicology and Teratology (2018), Vol. 67, pp. 44-49
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Classified as relevance cannot be determined (EFSA GD Point

as provided in the AIR5 dossier (KCA 9)	5.4.1 - category C)

2. Full summary of the study according to OECD format

As shown previously, glyphosate-based herbicides (GBH)-exposure has been shown to impact neurobehavioral functions and altered gut microbiota (GM) profiles. This has been associated with anxiety and depressive-like behaviour. It can be hypothesized that GBH-induced GM alterations may contribute to mediating behavioural changes. Thus, the purpose of the present study was to evaluate the impact of GBH on a healthy microbiota-gut-brain axis by investigating the gastrointestinal microbiota alterations and their subsequent effects on the neurobehavioral functions in mice.

In the conducted study, 18 male Swiss mice per dose were allocated into three experimental groups (acute, subchronic, and chronic). The mice assigned to the acute group received one administration of GBH, while the subchronic and chronic groups were treated daily for 6 and 12 weeks, respectively. Each experimental group was subdivided into three dose groups (0, 250, and 500 mg/kg/day GHB) consisted of 6 animals. These doses were selected based on glyphosate no-observed adverse effect level (NOAEL) of 500 mg/kg/day for subchronic toxicity. On the last day of the experiment, the treated animals were sacrificed for gut microbiota analysis.

The study revealed an exacerbation of anxiety and depression-like behaviours and significant perturbations in relative abundance and phylogenic diversity of gut microbiota in mice induced by GBH exposure.

Materials and methods

Test Material:	Roundup herbicide (glyphosate concentration 360 g/l in the form of glyphosate isopropylamine salt 486 g/l)
Origin:	Monsanto Company, St. Louis, MO, USA
Lot/Batch number:	Not reported
Purity:	Not reported
CAS#:	Not reported
approval	2020448
Vehicle:	NaCl 0.9%
Test Animals:	
Species	Mouse
Strain	Swiss mice
Sex	male
Age/weight on arrival	1 month old/Not reported
Source	Animal husbandry of the Faculty of Sciences, Cadi Ayyad University, Marrakech, Morocco.
Housing	Plexiglas cages (30 cm×15 cm×12 cm) Temperature (22 ± 2°C) Photoperiod 12 h/12 h (lights on at 08:00 h).
Acclimatisation period	Not reported
Diet	Not reported

Animals and treatment
Food and water were available *ad libitum*.

Eighteen male mice were divided to three experimental groups (acute, sub chronic and chronic) and subdivided in three dose groups (0, 250, and 500 mg/kg/day) with each 6 animals. Each group was subjected to orally gavages by NaCl 0.9% (control n = 6), by 250 mg/kg/day (n = 6) or 500 mg/kg/day (n = 6) of GBH.

These doses were selected based on glyphosate no-observed adverse effect level (NOAEL) of 500 mg/kg/day for subchronic toxicity (EPA, 1993).

The mice of the acute experimental group received one administration of 0.3 mL of GBH via gavage, while the subchronic and chronic groups were treated daily with 0.3 mL GBH for 6 and 12 weeks. After the treatment period, the animals were submitted to behavioural testing (open field, elevated plus-maze, tail suspension and splash test). On the last day of the experiment, the treated animals were sacrificed and their gut microbiota was analysed.

Open field test
The apparatus was a square field (50×50×50 cm), within each mouse was placed, and allowed to move freely for 20 min. The time spent in the centre area (15×15 cm), as an index of anxiety behaviour, was recorded using a video camera (JVC) and analysed by Ethovision XT Noldus 8.5 video tracking program (Noldus Information Technology b.v., Wageningen, Netherlands).

Elevated-plus maze test
Animals were tested individually for 5 min, by placing them in the centre of the maze platform, with the head facing an open arm. The time spent in the OA (open arms) and CA (closed arms) as well as the number of entries to each arm were quantified using Ethovision XT Noldus 8.5 video tracking program, allowing to evaluate the anxiety index expressed as:

$$\text{anxiety index} = 1 - \left(\frac{\frac{\text{OA time}}{\text{total time}} + \frac{\text{OA entries}}{\text{number of entries}}}{2} \right)$$

The tail suspension test
The mice were individually suspended by the tail above the ground, with adhesive tape placed about 40 cm from the floor. A single 6 min session was recorded for each animal. The total time spent immobile during the last 4 min of a session was scored.

Splash test
The latency to initiate a grooming behaviour, as well as the duration of grooming, was recorded during 5 min after the vaporization (10% sucrose solution) of sucrose solution. Previous works in mice have shown that in the splash test, chronic stress decreases grooming behaviour, a form of motivational behaviour considered to parallel indifferent behaviour as a symptom in depression.

Sample collection and abundances of intestinal microbiota determination
Thus, to examine the effects of a glyphosate-based herbicide on the composition of bacterial communities, intestinal samples were obtained from control and GBH-exposed mice at euthanasia, after behavioural tests completion. For each animal, a sample of the intestinal tract starting from the duodenum to the end of the large intestine was collected directly into a sterile tube, diluted ten times in sterile physiological water (NaCl 9 g/l), homogenized for 10-15 min. Bacterial strains were counted using a dilution/spreading method after the incubation at 37 °C for 72 h.

Phoenix system identification method
The Phoenix identification method uses modified conventional, fluorogenic, and chromogenic substrates. Combination panels for investigational use only (PMIC/ID-33, catalog no. 448587) for both identification and susceptibility testing were used for this comparison. Software V7. 00A/V5.91A was used for this study. The ID side contains 45 wells with dried biochemical substrates and 2 fluorescent control wells. The ID broth was inoculated with bacterial colonies adjusted to a 0.5 McFarland standard by using a Crystal Spec Nephelometer (BD Diagnostics), according to the manufacturer's recommendations. The specimen was logged and loaded into the instrument within the specified timeline of 30 min. Quality control and maintenance were performed according to the manufacturer's recommendations.

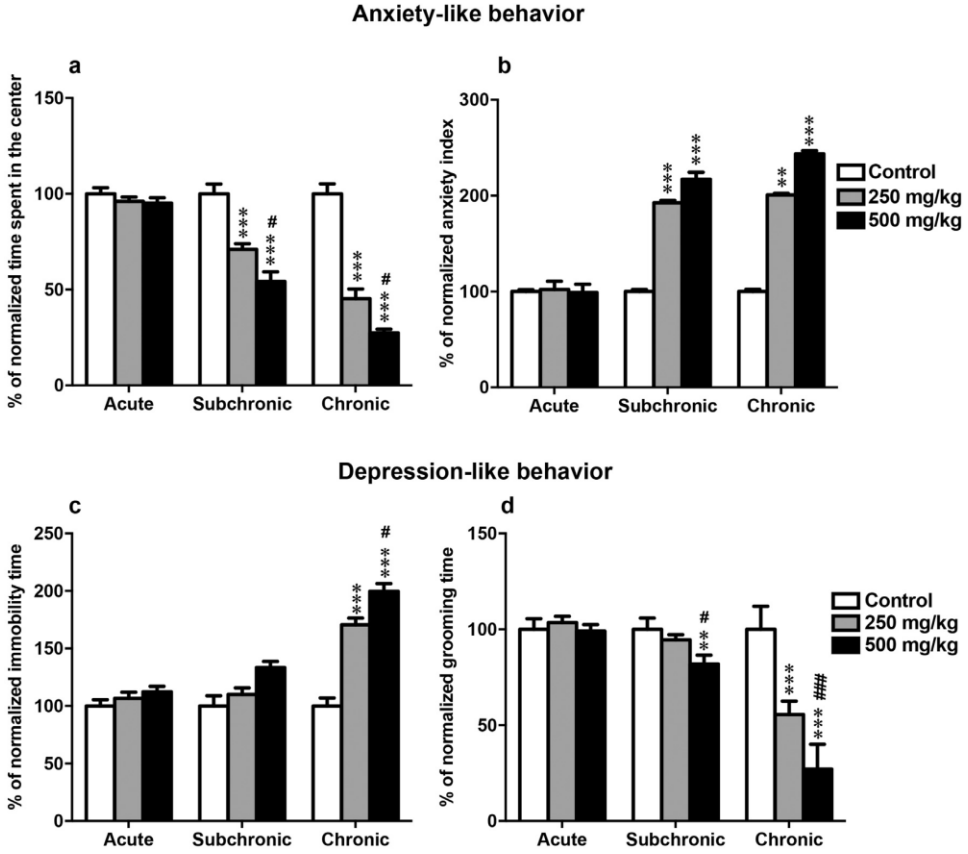
Statistical analysis
To compare data from behavioural tests and gut microbiota analysis between groups (control and treated), a statistical analysis of the different independent variables was performed by two-way ANOVA (treatment and treatment duration), using the Sigma Plot software 11.0. Post hoc analysis was performed using Holm-Sidak post hoc test.

Results are presented as mean ± standard error of the mean (S.E.M). The significance threshold was set at $p < 0.05$.

Results

Behavioural changes after GBH exposure
The results indicated that the control groups in each of the various tests (open field, elevated plus-maze, tail suspension, and splash test) showed no significant difference as a function of treatment duration, except for the controls of the open field test. A significant increase in controls of subchronic and chronic treatment groups compared to the acute control group was observed. The comparisons confirmed that the treated groups showed a significant increase in the immobility time only following chronic treatment and a decrease in the grooming time after both subchronic and chronic exposure.

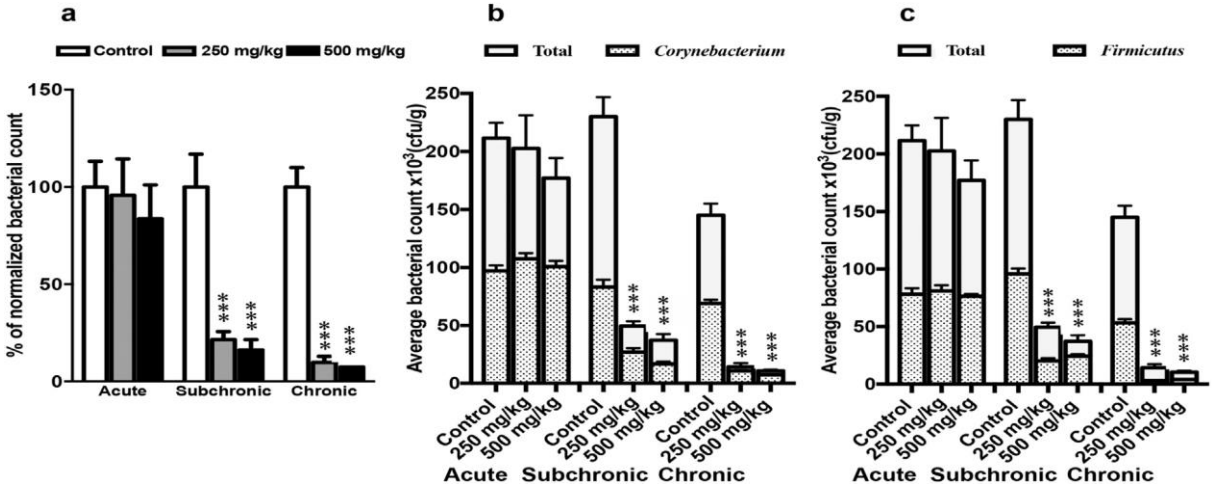
Figure 1: Effect of GBH on the anxiety and depression like-behaviours.
(a): Percentage of the time spent in the centre of open field test normalized to control.
(b): Percentage of the anxiety index in the elevated plus-maze test normalized to control.
(c): Percentage of the immobility time in the tail suspension test normalized to control.
(d): Percentage of the grooming time in the splash test normalized to control.
#P <0.05, ∗∗ and ##P <0.01, ∗∗∗P <0.001. The "∗" refers to the control vs. 250 mg/kg and 500 mg/kg group comparison, and the "#" refers to the 250 mg/kg vs. 500 mg/kg group comparison.



Gut microbiota changes after GBH exposure

It was shown that the repeated exposure to GBH acted significantly on GM profile of exposed mice. However, GM decreased significantly in the control of chronic treatment group compared to both acute control group and subchronic treatment control group.

Figure 2: Effect of GBH on the intestinal bacteria abundance.
(a): Percentage of the total bacterial count normalized to control group.
(b): *Corynebacterium* strain count.
(c): *Firmicutus* strain count.
***P <0.001. "*" refers to the control vs. 250 mg/kg and 500 mg/kg group comparison.



Gut microbiota diversity after GBH exposure

The identified gut bacteria assigned at the intestine level showed that control mice were dominated by *Firmicutes*, *Bacteroidetes*, *Corynebacterium* and *Lactobacillus* species, while the GBH treated mice were

dominated only by *Firmicutes* and *Corynebacterium* species following subchronic and chronic exposure. Furthermore, the abundance of *Corynebacterium* was significantly affected under GBH exposure. It could be shown that *Corynebacterium* and *Firmicutus* decreased statistically significantly for both treated groups (250 and 500 mg/kg), compared to their controls within subchronic and chronic treatments. However, no significant difference between the treated groups was noted.

Table 1. Effect of GBH on taxonomic diversity of gastrointestinal microbiota

Type of bacteria acute treatment				Subchronictreatment			Chronictreatment		
	Control	250 mg/kg	500 mg/kg	Control	250 mg/kg	500 mg/kg	Control	250 mg/kg	500 mg/kg
Firmicutes spp.	+	+	+	+	+	+	+	+	+
Coryenobacterium spp.	+	+	+	+	+	+	+	+	+
Bacteroidetes spp.	+	+	+	+	−	−	+	−	−
Lactobacillus spp.	+	+	+	+	−	−	+	−	−

(+): presence, (−): absence.

Conclusion

The conducted study found that the anxiogenic and depressive-like behaviours observed in the present work paralleled by altered gut microbiota in GBH-treated mice in term of abundance and bacteria species. Indeed, the oral exposure to GBH decreased the gut microbiota abundance of *Firmicutes*, *Corynebacterium*, *Bacteroidetes* and *Lactobacillus.*

In this study, it was concluded that an exacerbation of anxiety and depression-like behaviours and significant perturbations in relative abundance and phylogenic diversity of gut microbiota in mice were induced by GBH exposure. Nevertheless, it should be kept in mind that non-cultivatable bacterial communities of the microbiome were not investigated. The toxicity of Roundup and the effect on gut microbiome could be either due to Glyphosate or its formulants, or to a synergistic effect of all components.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case c) Relevance cannot be determined: Potential effects on gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus the relevance of the effects remained unclear. This study uses Roundup administered at half of or at the NOAEL concentration via a stomach tube. The surfactant is irritating and any negative results are not surprising. The acidic effect of glyphosate is also a concern. Non-cultivatable bacterial communities of the microbiome were not investigated and the effects of surfactants in the used product were not taken into account.
No batch, purity or CAS No. of the test substance reported. Diet and acclimatisation period of animals not mentioned. No positive control used. No historical control data are given.

The exact composition of the Roundup formulation tested is not stated in the paper (a.i. content, source, surfactant system / co-formulants). It is therefore not possible to confirm whether the product used is the representative glyphosate formulation MON 52276 relevant for the glyphosate EU renewal. Furthermore, in the absence of a concurrent control for each to the component of the formulation, it is not possible to conclude whether the observed effects claimed to be secondary to exposure to glyphosate are due to glyphosate exposure or of one of the other components. The uncertainty associated with whether the product contains polyethoxylated tallow amine (also polyoxyethyleneamine, POEA) or not, suggests that the findings in this paper should be treated with high level of caution.

Assessment and conclusion by RMS:
In this study the effects of glyphosate-based herbicides on gut microbiota and its subsequent effects on the neurobehavioral functions in mice is investigated. Male Swiss mice were exposed to either 250 mg/kg/day or 500 mg/kg/day as a single dose (acute group), for 6 weeks daily (sub-chronic group) or daily for 12 weeks (chronic group). The dose was not corrected for bodyweight of the animals. Then the animals were submitted to behavioural testing (open field test, elevated-plus maze test, tail suspension test and splash test) just before the treated animals were sacrificed for gut microbiota analysis. Effects of glyphosate-based herbicides were seen on neurobehavioral functions, increasing anxiety and depression-like behaviour, and on gut microbiota, reducing the levels of *Firmicutes*, *Corynebacterium*, *Bacteroidetes* and *Lactobacillus*.

The neurobehavioral part of the study is considered unreliable by the RMS considering the large number of deviations from OECD 424 and the fact that the study was not conducted according to GLP. In addition to the deviations already mentioned, the number of animals per dose is only 6 instead of the recommended 10, only male animals are used, only two doses were used instead of the recommended three, the dose is not corrected for the bodyweight of the animals, only a single observation of behavioural effects is made only at the end of the dosing period, no information on general health condition and/or mortality/morbidity is given. It is not stated if the observations were made by trained observes unaware of the actual treatment, bodyweights of the animals were not recorded nor were neuropathological alterations investigated. Only the normalized and not the individual data is reported. Moreover, the study is carried out with a formulation of glyphosate, thus effects caused by co-formulants cannot be excluded.

Since investigation of the gut microbiota is currently not part of the European assessment framework for pesticides, it is hard to assess the relevance of the findings. The RMS notes that there is an absence of a clear conceptual link between the effects on the mice gut microbiota and the neurobehavioral functions. On the aspect of effects on the gut microbiome, the study is not directly relevant for the risk assessment.

B.6.8.2.19. Public literature – MIC of glyphosate and formulation for E. coli isolates (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.8
Report author	Bote K. *et al.*
Report year	2019
Report title	Minimum inhibitory concentration of glyphosate and of a glyphosate containing herbicide formulation for Escherichia coli isolates - Differences between pathogenic and non-pathogenic isolates between host species.
Document source	Frontiers in microbiology (2019), Vol. 10, pp. 932
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevance cannot be determined (EFSA GD Point 5.4.1 - category C)

2. Full summary of the study according to OECD format

This study analyses a large set of recent and historical *Escherichia coli* isolates varying in pathogenicity and beta-lactam resistance from different host species for their susceptibility to glyphosate isopropylamine salt (IPA), the active ingredient of the herbicide, and to a complete glyphosate-containing formulation (Roundup LB Plus). For this, minimum inhibitory concentrations (MIC) were determined for 238 *E. coli* isolates by broth microdilution in Mueller Hinton I media followed by the statistical analyses using Mann-Whitney-U test, multivariable analysis of variance (ANOVA) and a multivariable proportional-odds ordinal regression model. While the overall MIC distribution was narrow and lacked a highly resistant sub-population for both substances, statistical analyses revealed small but significant associations between glyphosate resistance levels and different factors tested. Mean MIC values for the entire dataset showed a higher level of resistance to the complete glyphosate-containing formulation (40 mg/mL IPA) than to pure glyphosate (10 mg/mL IPA) in *E. coli*. Isolates that originated from poultry had significantly higher MIC values for both pure glyphosate and the complete formulation. Pathogenic and non-extended-spectrum beta-lactamase (non-ESBL) *E. coli* isolates each showed significantly higher MIC values compared to commensals and ESBL-producing *E. coli* in pure glyphosate, but not in the complete formulation. Recently sampled isolates showed statistically higher MICs than the isolates of the historic standard *E. coli* collection of reference in pure glyphosate, when tested by nonparametric Mann-Whitney-U test, but not in the multivariable model. Further investigations are necessary to confirm whether these associations have a causal relationship with the glyphosate use or are the consequence of co-selection due to the increased application rates of antibiotics, heavy metals or other biocides. A possible accumulation of pathogenic bacteria in livestock animals fed with glyphosate-containing feed should also be considered.

Materials and methods

Test Material:		40 % monoisopropylamine salt solution of glyphosate (I) Roundup LB Plus (RU) (II)
	Origin:	Sigma-Adrich Chemie GmbH, Taufkirchen, Germany (I) Not reported (II)
	Lot/Batch number:	Not reported
	Purity:	Monoisopropylamine salt: 40% Roundup LB Plus: Not reported
	CAS#:	024142-00 (II)
Vehicle:		Mueller Hinton I media
Concentration of test items:		Glyphosate isopropylamine salt: 80 to 1.25 mg/mL Roundup LB Plus: 160 to 2.5 mg/mL

Test system
Table 1 shows the different *E. coli* isolates that were used for the determination of the minimum inhibitory concentrations (MIC) of glyphosate and its formulation Roundup LB Plus.

65 *E. coli* isolates from the standard *E. coli* collection of reference (ECOR) were used as historical controls, as this collection was established before the broad usage of glyphosate, thus representing the variations in *E. coli* at that time.

90 of the *E. coli* isolates were obtained from the German Federal Institute for Risk Assessment and were sampled from 2014 to 2015.

83 pathogenic *E. coli* isolates were obtained from the German Federal Office of Consumer Protection and Food Safety which were collected in 2014 and 2015 as part of the GERMAP survey.

Minimum inhibitory concentration (MIC) testing
For the testing of MICs of glyphosate, a susceptibility testing protocol was established, which complied with CLSI M07-A10 standards for antibiotic susceptibility testing.

Pure glyphosate (GLY) and Roundup LB Plus (RU) were diluted with Mueller Hinton (MH) I medium resulting in a concentration ranging from 80 to 1.25 mg/mL and 160 to 2.5 mg/mL, respectively.

For testing, overnight cultures were diluted to an OD_{600} of 0.5 (10^8 cfu/mL), which were further diluted 1:100 before adding 5 mL into each well (equivalent to 5×10^4 cfu, 5×10^5 cfu/mL, respectively) of a polystyrene 96-well plate with a conical bottom. Each isolate was tested in triplicates. The plates were aerobically incubated at 37°C for 16–20 h in a humidity chamber. The growth within the wells was determined visually with a mirror below the plate and a light above (SensiTouch by Sensititre).

Statistical analysis

For statistical analyses and calculations, IBMR SPSSR Statistics Version 24 was used. All MIC data were ranked in ascending order prior to analyses and checked visually for normal distribution. As MIC values of GLY showed sufficient normal distribution, the data of GLY could be fitted by an ANOVA approach. Regarding RU, the MIC values were not normally distributed and only included the three levels 20, 40, and 80. Thus, it was decided to regard these levels as ordinal categories and to fit a proportional-odds ordinal regression model. The influences in terms of isolation time (ECOR and recent isolates), collection (commensals and pathogens), ESBL-status and host (poultry, pig, cattle) on MIC values of GLY or RU, respectively, were tested using:

(i) univariable nonparametric Mann-Whitney-U tests for not normally distributed data, and
(ii) a multivariable analysis of variance (ANOVA) for GLY, or
(iii) a multivariable proportional-odds ordinal regression model for RU to determine different factors.

Two different statistical models for each substance were adapted containing different parameters. In the first model (Model A) the influence of the time of isolation, the ESBL-status and the host on either GLY or RU were investigated. In the ECOR collection, there were only a few livestock-associated isolates (two *E. coli* from pigs and three from cattle). Most of the isolates originated from humans or exotic animals (Table 1). Therefore, we created a second model (Model B) without the ECOR collection, which investigated the influence of the collection (pathogen or commensal), the ESBL-status and the host (poultry, pig, cattle) on either GLY or RU. All two-way-interactions between influence factors were included in the initial models and removed if not statistically significant. *P*-values below 0.05 were regarded as statistically significant. Model diagnostics included a check for normality and homoscedasticity of residuals. For analysis of variance, the assumption of equal variances was also investigated. For proportional odds ordinal regression models, the assumption of proportionality as well as the assumption of parallel lines were additionally checked. To obtain an epidemiological cut-off, MIC95 was calculated for GLY and RU each.

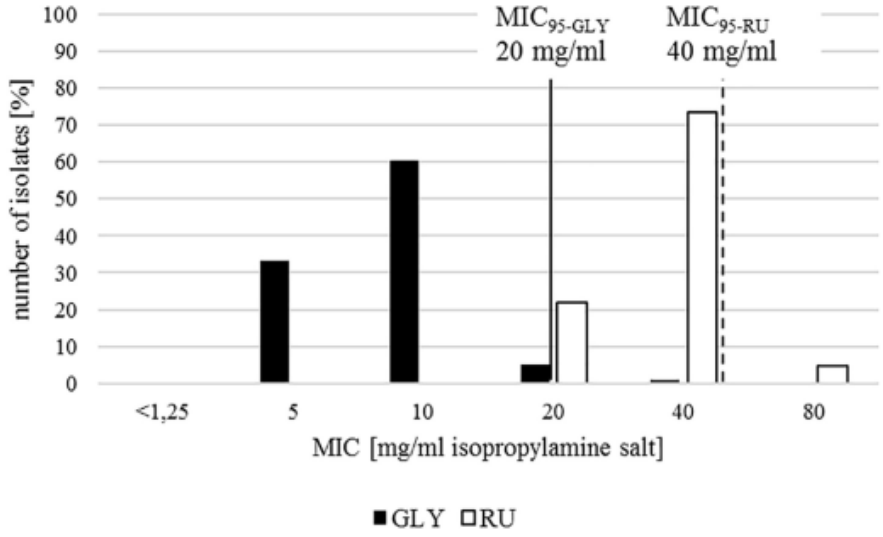
Table 1. Origin and distribution of the 238 tested *E. coli* isolates divided by different collections.

Origin	ECOR	Commensal *E. coli*			Pathogenic *E. coli*		
	Non-ESBL	ESBL	Non-ESBL	in total	ESBL	Non-ESBL	in total
Poultry	–	15	15	30	3	12	15
Pig	2	15	15	30	19	17	36
Cattle	3	15	15	30	15	17	32
Human	39	–	–	–	–	–	–
Primate	9	–	–	–	–	–	–
Dog	3	–	–	–	–	–	–
Sheep	2	–	–	–	–	–	–
Leopard	2	–	–	–	–	–	–
Bison	1	–	–	–	–	–	–
Giraffe	1	–	–	–	–	–	–
Goat	1	–	–	–	–	–	–
Cougar	1	–	–	–	–	–	–
Kangaroo Rat	1	–	–	–	–	–	–
in total	65	45	45	90	37	45	83

Results

Figure 1 shows the MICs of GLY and RU in all 238 isolates. In most of them, growth was inhibited at a concentration of 10 mg/mL GLY (equal to 7.41 mg/mL pure glyphosate) or 40 mg/mL RU (equal to 29.63 mg/mL pure glyphosate), both representing the mean and the mode.

Figure 1. *In vitro* susceptibility profile of 238 *E. coli* isolates for glyphosate isopropylamine salt in pure solution (GLY, black) and Roundup LB Plus (RU, white). Minimum inhibitory concentration including 95% of all isolates (MIC_{95}) is represented with a continuous line for GLY and a dashed line for RU.



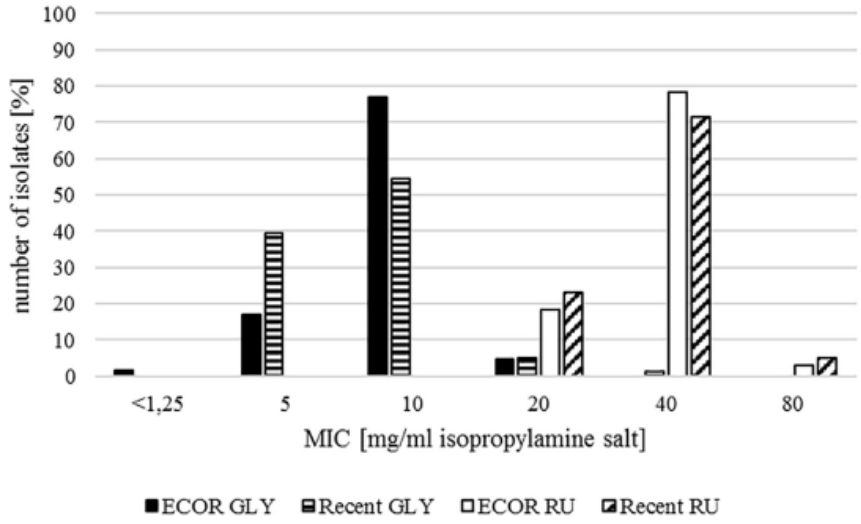
Most of the isolates from the ECOR collection showed a MIC of 10 mg/mL for GLY, which represented the mode and the median. For the herbicide formulation RU, the majority of the isolates had MIC values of 40 mg/mL. Overall MICs ranged from <1.25 to 20 mg/mL for GLY and 20 to 80 mg/mL for RU (Table 2 and Figure 2).

Table 2. MIC values of 238 *E. coli* for monoisopropylamine salt (IPA) represented either as a pure solution (GLY) or as a part of the complete formulation Roundup LB Plus (RU).

MIC [mg/ml IPA]	ECOR		recent isolates		Commensal *E. coli*		Pathogenic *E. coli*		ESBL		non-ESBL		*E. coli* in total	
	GLY	RU	GLY	RU	GLY	RU	GLY	RU	GLY	RU	GLY	RU	GLY	RU
<1,25	1.5% (1)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0.7% (1)	0% (0)	0.4% (1)	0% (0)
5	16.9% (11)	0% (0)	39.3% (68)	0% (0)	48.9% (44)	0% (0)	28.9% (24)	0% (0)	45.8% (38)	0% (0)	26.5% (41)	0% (0)	33.2% (79)	0% (0)
10	76.9% (50)	0% (0)	54.3% (94)	0% (0)	47.8% (43)	0% (0)	61.5% (51)	0% (0)	51.8% (43)	0% (0)	65.2% (101)	0% (0)	60.5% (144)	0% (0)
20	4.6% (3)	18.5% (12)	5.2% (9)	23.1% (40)	3.3% (3)	22.2% (20)	7.2% (6)	24.1% (20)	2.4% (2)	24.1% (20)	6.5% (10)	20.7% (32)	5.0% (12)	21.9% (52)
40	0% (0)	78.5% (51)	1.2% (2)	71.7% (124)	0% (0)	74.4% (67)	2.4% (2)	68.7% (57)	0% (0)	73.5% (61)	1.3% (2)	73.6% (114)	0.8% (2)	73.5% (175)
80	0% (0)	3.1% (2)	0% (0)	5.2% (9)	0% (0)	3.3% (3)	0% (0)	7.2% (6)	0% (0)	2.4% (2)	0% (0)	5.8% (9)	0% (0)	4.6% (11)

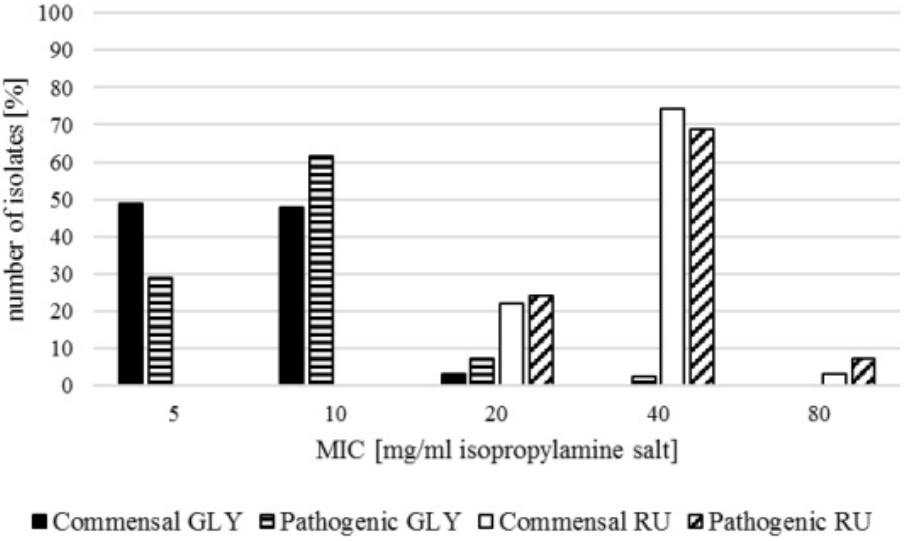
The tested isolates were divided into different groups. The ECOR collection served as an example of historic isolates as opposed to recent isolates (consisting of commensal and pathogenic isolates gathered in 2014 and 2015) or separated according to the susceptibility against beta-lactam antibiotics. Indicated as percentage share rounded to one decimal place after the point with the number of isolates in brackets.

Figure 2. MIC for IPA for the ECOR collection (ECOR GLY, black) and the recently sampled isolates (Recent GLY, black with white stripes) and for the formulation RU for the ECOR collection (ECOR RU, white) and the recently sampled isolates (Recent RU, white with black oblique stripes), respectively.



The commensal isolates of the investigated strains showed mostly a MIC of 5 mg/mL (representing the mode) or 10 mg/mL (representing the median) for GLY with a total range from 5 to 20 mg/mL. RU inhibited the growth of most strains at 40 mg/mL with a total range from 20 to 80 mg/mL (Table 2 and Figure 3). In contrast to commensal isolates, pathogenic *E. coli* mostly showed a MIC of 10 mg/mL for GLY with a total range of 5-40 mg/mL. For RU, the MIC was in the range of 20–80 mg/mL, whereby 40 mg/mL was the most common minimal inhibitory concentration (Table 2 and Figure 3). MIC95 representing 95% of the studied population was 20 mg/mL in GLY and 40 mg/mL in RU. For GLY there are two pathogenic *E. coli* isolated from cattle with a higher MIC than the cut-off. For RU 11 isolates (two from the ECOR collection isolated from humans, three commensal and five pathogenic *E. coli* from poultry and one pathogenic isolate from a pig) showed a MIC above the MIC95. All of the isolates belong to the non-ESBL group.

Figure 3. MIC for IPA for the commensal *E. coli* (Commensal GLY, black) and the pathogenic *E. coli* isolates (Pathogenic GLY, black with white stripes) and for the formulation RU for the commensal *E. coli* (Commensal RU, white) and the pathogenic *E. coli* isolates (Pathogenic RU, white with black oblique stripes), respectively.



Statistical analysis

To test for differences between isolate parameters in glyphosate sensitivity, nonparametric Mann-Whitney-U test and depending on data distribution, two different statistical models were used. In the Mann-Whitney-U test, both for GLY and RU, there were highly significant differences in MICs between the isolates from poultry (P <0.01) compared to pig and cattle isolates which had lower MICs (Table 3).

Table 3. Effect of different parameters on MIC by means of univariable nonparametric Mann-Whitney-U test.

Comparison of		*P*-value	
		GLY	RU
Recent isolates	Historic isolates	**0.014**	0.667
ECOR collection	Commensal *E. coli*	**<0.001**	0.623
ECOR collection	Pathogenic *E. coli*	0.498	0.780
Pathogenic *E. coli*	Commensal *E. coli*	**0.004**	0.861
Non-ESBL	ESBL	**0.018**	0.362
Poultry	Pig	**<0.001**	**0.004**
Poultry	Cattle	**0.001**	**0.007**
Pig	Cattle	0.078	0.627

Statistically significant P-values <0.05 are in bold. Parameters with higher MICs are underlined.

Furthermore, more factors showed significant influence on GLY. Historic isolates from the ECOR collection had significantly lower MIC values (P <0.05) than the isolates collected in the years 2014 and 2015. Pathogenic isolates differed highly significantly (P <0.01) from the commensal isolates (with higher MIC values in the pathogenic group). Likewise, isolates classified as non-ESBL had statistically significantly higher MICs than the ESBL isolates (P <0.05). Model A included the time of isolation (historic and recent), ESBL-status and host, whereas Model B (with the excluded ECOR strains) considered isolation as commensal or pathogen, ESBLstatus and host (Table 4).

Table 4. P-values of the statistical models for GLY (multivariable analysis of variance) and in RU (multivariable proportional-odds ordinal regression).

GLY		*P*-value		RU		*P*-value	
Comparison of		Model A	Model B	Comparison of		Model A	Model B
Recent isolates	Historic isolates	0.726	–	Recent isolates	Historic isolates	0.293	–
Pathogenic *E. coli*	Commensal *E. coli*	**–**	**<0.001**	Pathogenic *E. coli*	Commensal *E. coli*	**–**	0.314
Non-ESBL	ESBL	**0.013**	**0.035**	Non-ESBL	ESBL	0.443	0.479
Poultry	Pig	**<0.001**	**<0.001**	Poultry	Human	**0.031**	**–**
Poultry	Cattle	**0.01**	**0.002**	Poultry	Pig	**–**	**0.001**
Pig	Cattle	0.608	0.229	Poultry	Cattle	–	**0.002**

Model A investigates the time point of isolation, the ESBL-status and the host, Model B (without the ECOR collection) investigates the following categories: pathogenic or commensal, the ESBL-status and the hosts (poultry, pig, and cattle). Statistically significant P-values <0.05 are shown in bold. Group with higher minimum inhibitory concentrations are underlined.

In contrast to the results of the Mann-Whitney-U test for GLY, no difference between the strains of the ECOR collection and recently sampled isolates was seen in model A (P = 0.726). However, the ESBL-status and the host species of the isolates showed a statistically significant influence on the MIC values (P = 0.013 and P <0.001). In agreement with the Mann-Whitney-U test, non-ESBL isolates had significantly higher MIC values compared to ESBL-positive isolates. Tukey *post hoc* analysis for the hosts revealed significant differences between isolates from poultry and pigs (P <0.01) and poultry and cattle (P = 0.01) with higher MICs in the poultry each, as well as between isolates from pigs and human (P = 0.019) and pigs and other species (P = 0.006) with lower MICs in pigs each. There was no significant difference between the isolates from pigs and cattle (P = 0.608). Model B classified the differences between ESBL and non-ESBL isolates (P = 0.035) as well as between the hosts as significant. In accordance with model A, non-ESBL isolates and isolates from poultry had significantly higher MIC values than the ESBL isolates and isolates from cattle and pig. Additionally, a significant interaction between pathogenic and commensal isolates was present (P <0.001). Pathogenic *E. coli* isolates showed significantly higher MIC values than commensals. In the *post hoc* analysis,

the differences between poultry and cattle ($P = 0.002$) as well as between poultry and pigs ($P < 0.001$) were clearly visible, with *E. coli* isolates from poultry showing significantly higher MIC values for glyphosate than isolates from other hosts. For RU in model A, there was no significant difference between the strains of the ECOR collection and recently sampled isolates ($P = 0.293$), nor between ESBL and non-ESBL isolates ($P = 0.443$). However, a significant difference was found between poultry and human isolates (human isolates served as a reference category, $P = 0.031$). The Nagelkerke R^2 in this model was 0.088, meaning that only a small proportion of the variance could be explained with this model. Model B also showed no significant differences between ESBL and non-ESBL ($P = 0.479$) nor between commensal and pathogenic isolates ($P = 0.314$). Nevertheless, there was a significant difference between the hosts, i.e., between cattle and poultry ($P = 0.002$) and pigs and poultry ($P = 0.001$). Poultry served as reference category and had the highest MIC values compared to cattle and pigs. With a Nagelkerke's R^2 of 0.111, it still only explained a small proportion of the variance. Obviously, the variables included in the model were not the most important influence factors on the MIC values of the investigated *E. coli* strains.

Conclusion

In conclusion, the authors conducted a large-scale screening for GLY and RU susceptibilities in 238 isolates of *E. coli*. We found small but statistically significant differences between the tested formulation RU and the pure glyphosate salt as well as between poultry and other host animals with higher MIC values in Roundup and poultry. Furthermore, for glyphosate, we observed differences between non-ESBL and ESBL, and between pathogenic and commensal isolates (higher MICs in the former group). The difference between recently sampled isolates and the historic ECOR collection from 1984 was only found when the Mann-Whitney-U test for GLY was applied, but this finding was not confirmed by the modelling.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus the relevance of the effects remained unclear. The study uses a system designed to measure antibiotic MICs that are usually done by culturing bacteria in a specific media for antibiotic diffusion in µg/mL range. Instead, the paper looks at glyphosate in mg/mL range following MIC procedures. There is no justification for the dose, which should be at about 100000X lower dose. Most gut microbes are anaerobes.

Origin and purity of Roundup LB Plus is not reported.

The exact composition of the Roundup formulation tested is not stated in the paper (a.i. content, source, surfactant system / co-formulants). However, the data on the formulation are not relevant for the glyphosate EU renewal (formulation used is not the representative formulation MON 52276). Furthermore, in the absence of a concurrent control for each of the component of the formulation, it is not possible to conclude whether the observed effects claimed to be secondary to exposure to glyphosate are due to glyphosate exposure or to one of the other components.

Further points for clarification:
The study investigates the susceptibility of in total 238 *E. coli* isolates, obtained from different species, to pure glyphosate and its formulation (Roundup LB Plus). Therefore, the isolations were exposed to a range of concentrations of pure glyphosate or its formulation and the minimum inhibitory concentrations (MIC) were determined.

Overall, the results suggest that the isolates were more resistant to the glyphosate formulation with a MIC of 40 mg/mL compared to pure glyphosate with a MIC of 10 mg/mL. However, different MICs were observed in isolates from different species.

Assessment and conclusion by RMS:
In this study the effects of 40% monoisopropylamine salt solution of glyphosate and the glyphosate-containing commercial formulation Roundup LB Plus (German registration number 024142-00) on *E.coli* was investigated by screening different *E.coli* isolates of clinical, non-clinical and environmental origin for susceptibility and compare historical and recent isolates in regards to the development of resistance over time and lastly investigate whether there is a link between host species or antibiotic resistance and glyphosate susceptibility. In total 238 *E.coli* strains were analysed for the minimum inhibitory concentration. 65 were categorized as historic controls as they were collected before the broad usage of glyphosate, 90 were categorized as non-pathogenic and divided into poultry, pig, cattle, extended spectrum beta-lactamase and non-extended spectrum beta-lactamase and lastly 83 were categorized as pathogenic *E.coli* isolates from clinical cases. The study results show a small but significantly higher minimum inhibitory concentration for Roundup and poultry. For glyphosate a difference between non-ESBL and ESBL, and between pathogenic and commensal isolates were observed, with higher minimum inhibitory concentrations in the former group. No difference was found between the historic and recently sampled isolates.

Since investigation of the gut microbiota is currently not part of the European assessment framework for pesticides, it is hard to assess the relevance of the findings. It is agreed with the conclusions by the applicant. Additionally, it is unclear, as also stated in the study report, if the isolates from the recent hosts were previously exposed to glyphosate in the intestine of the host or the environment as data about residues in feed are missing.

B.6.8.2.20. Public literature – Glyphosate suppresses antagonistic effect enterococcus spp. on Clostridium botulinum (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.8
Report author	Kruger M. *et al*.
Report year	2013
Report title	Glyphosate suppresses the antagonistic effect of *Enterococcus* spp. on *Clostridium botulinum*
Document source	Anaerobe (2013), Vol. 20, pp. 74-78
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevance cannot be determined (EFSA GD Point 5.4.1 - category C)

2. Full summary of the study according to OECD format

During the last 10-15 years, an increase of *Clostridium botulinum* associated with acute and chronic diseases in cattle has been observed in Germany. The reason for this development is currently unknown and the factors that determine the severity of the disease and the factors that prevent intestinal colonisation by *C. botulinum* spores are incompletely characterized.

In the mouse model of infant botulism, it could be shown that the normal intestinal, enteric microflora is a critical factor in preventing intestinal colonisation by *C. botulinum* and other pathogens, as well as the microflora is able to protect the host from bacterial toxins, for example, botulinum neurotoxin (BoNT), by the production of lipids and gangliosides. An additional protection system is the production of bacteriocines by numerous bacteria in the gastrointestinal tract (GIT). This way bacteria, like lactobacilli, lactococci and enterococci, are generating bacteriocins in form of lactic acid (LAB), which were shown to be effective against *Clostridium* spp.

A reduction of LAB in the GIT microbiota by the ingestion of biocides, which are used as herbicides but are additionally able to act as microbiocides, as for example, Glyphosate (N-(phosphonomethyl) glycine), could be an explanation for the observed increase in levels of *C. botulinum* associated diseases in cattle.

Materials and methods

Test Material:	Roundup UltraMax which contains 450 mg/mL of glyphosate
Origin:	Monsanto, USA.
Lot/Batch number:	Not reported
Purity:	Not reported
CAS#:	Not reported
	N-(phosphonomethyle)glycine
Origin:	Sigma Aldrich, Taufkirchen, Germany
Lot/Batch number:	Not reported
Purity:	Not reported
CAS#:	Not reported

Isolation and identification of Enterococcus strains
Enterococcal isolates were isolated from cattle, horses, and algae (*Chlorella vulgaris*), by plating specimens on citrate- acid-tween-carbonate and incubated aerobically at 37 °C for 48 h. Typical red colonies were sub-cultured on Caso agar (3.5% Casein-Soya, 0.3% yeast extract, 0.1% glucose, 1.5% Agar Agar) and examined for Gram reaction and cellular morphology. Gram-positive, catalase-negative cocci isolated on this medium were presumptively identified as *Enterococcus* spp. Species identification of isolated strains was based on their matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) profile. Before each MALDI run, *E. coli* 1917 strain Nissle (Ardeypharm GmbH, Herdecke, Germany) was analysed to serve as the positive control and calibration standard.

C. botulinum strains

Strain:	**Supplier:**
C. botulinum type A (7272)	National collection of type culture (NCTC)
C. botulinum type B (7273),	National collection of type culture (NCTC)
C. botulinum type C (8264)	National collection of type culture (NCTC)
C. botulinum type E (8266)	National collection of type culture (NCTC)
C. botulinum type D (Pasteur 1873-D)	Institute of Pasteur, Paris, France

C. botulinum cultivation
C. botulinum strains were cultured anaerobically in a cooked meat medium at 37°C for 5 d. The cultivation is followed by cultivation in reinforced clostridial medium anaerobically at 37°C for 3 d. *C. botulinum* types A and B were heated at 80°C for 10 min, while types C, D and E were heated at 60°C for 30 min and left aerobically at room temperature. Cultures were analysed daily for sporulation using a Gram or Rakette stain.

Effect of Enterococcus on C. botulinum types A, B, C, D, and E
To study the effect of *Enterococcus* spp. on *C. botulinum* strains, heat-treated spores or vegetative cells were added to RCM medium at a final concentration of 10^4 cfu/mL. The inhibitory effect of different *Enterococcus* spp. was studied by the addition of different bacterial dilution (10^1-10^9 cfu/mL to *C. botulinum* culture medium. *C. botulinum* was quantified using the most probable number (MPN) estimation method using Differential Reinforced Clostridial broth, and the neurotoxins were tested using an ELISA.

Inhibitory activity of glyphosate on Enterococcus isolates
Inhibitory and bactericidal activity Minimal inhibitory concentration tests (MIC) and minimal bactericidal concentration tests (MBC) were done with glyphosate. The lowest concentration of glyphosate and Roundup, to show bactericidal or bacteriostatic effects, was determined in a 96-well micro-titre plate. Serial dilutions of glyphosate (from 10 to 0.001 mg/mL were made in nutrient broth. *Enterococcus* isolates were added at a final concentration of 10^4 cfu/mL, and the test plates containing diluted glyphosate and *Enterococcus* were incubated overnight at 37°C before plating aliquots on CATC agar. Bacterial growth on each agar plate was evaluated.

Kinetics of the inhibitory effect of glyphosate on Enterococcus faecalis
To study the effect of glyphosate and Roundup on *E. faecalis*, cells were added to RCM medium at a final concentration of 10^4 cfu/mL. The inhibitory effects of different glyphosate concentrations were studied after 8 h cultivation at 37°C under anaerobic conditions.

Results

Inhibition of BotNT production by isolated Enterococus spp.
All tested *Enterococcus* spp, isolated from *Chlorella vulgaris* and from faeces of cattle and horses inhibited neurotoxin production by *C. botulinum* types A, B, D and E reference strains as shown in following Table 1.

Table 1. Effect of different *Enterococcus* spp. on neurotoxin production in a co-culture with *C. botulinum* types A, B, D and E.

Enterococcus spp.[a]	Lab. designation	Source	Neurotoxin determination by ELISA[b]			
			BotNt A	BotNt B	BotNt D	BotNt E
E. faecium	E1	*Chlorella vulgaris*	–	–	–	–
E. fecalis	E1	*Chlorella vulgaris*	–	–	–	–
E. faecium	E3	Cattle	–	–	–	–
E. hirae	E4	Cattle	–	–	–	–
E. faecalis	E5	Horse	–	–	–	–
E. malomaoldoratus	E6	Horse	–	–	–	–
E. derrisei	E7	Horse	+/–	–	–	+
E. durans	E8	Cattle	–	–	–	–
E. faecium	E9	Cattle	–	–	–	–
E. feacalis	E10	Cattle	–	–	–	–
E. faecalis	E11	Cattle	–	–	–	–
E. faecalis	E12	Horse	+/–	–	–	–
E. hirae	E13	Horse	–	–	–	–
E. casseliflavus	E14	Cattle	+/–	–	–	–
E. coli strain (Nissle 1917)			++	++	++	++
–			++	+++	++	++

Enterococcus spp. prevent toxin production of *C. botulinum* types A, B, D and E while *E. coli* 1917 strain Nissle has no effect.
[a] Species identification performed by MALDI-TOF.
[b] ELISA results expressed as negative (–), low positive (+), positive (++) and highly positive (+++) compared with standards *C. botulinum* neurotoxins with known concentrations.

Cell growth of C. botulinum species in co-culture with Enterococcus spp.
C. botulinum type C did not produce neurotoxin. Likewise, all enterococci co-cultivated with *C. botulinum* reduced the growth (cell numbers) of *C. botulinum* type A, B, C, D and E (Table 2).

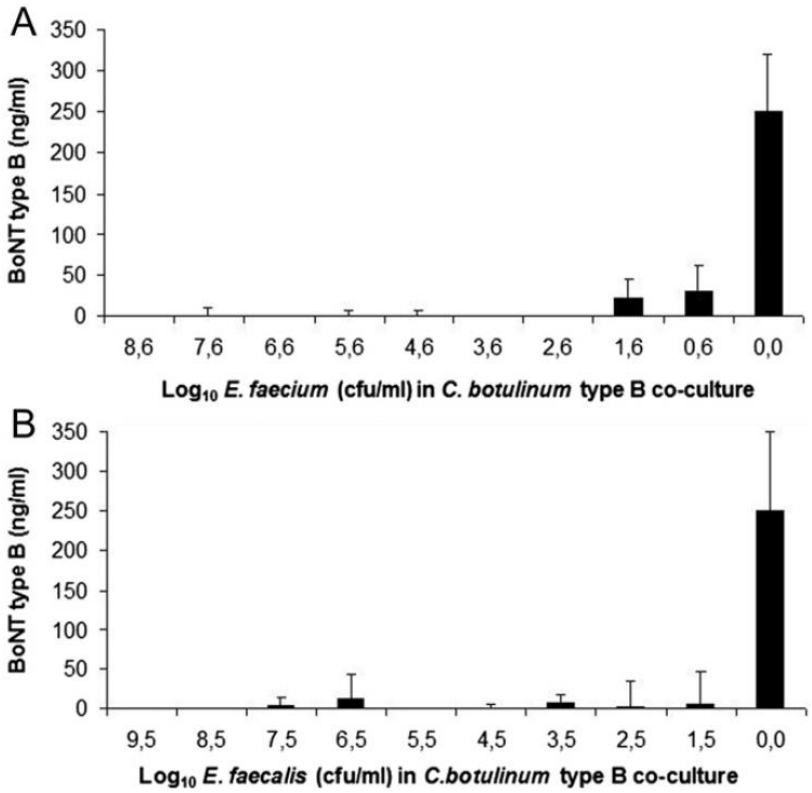
Table 2. Effect of different *Enterococcus* spp. on the growth of *C. botulinum* types A, B, C, D and E incubated anaerobically at 37°C for 5 days.

Enterococcus spp.	Log_{10} cell counts of *C. botulinum*[a] (Mean ± SD, $n = 3$)				
	Type A	Type B	Type C	Type D	Type E
E1	2.24 ± 0.49	2.75 ± 0.68	3.10 ± 0.22	3.04 ± 0.15	3.08 ± 0.42
E2	2.32 ± 0.63	3.02 ± 0.19	3.70 ± 0.36	2.99 ± 0.21	2.37 ± 0.10
E3	2.68 ± 0.39	3.52 ± 0.35	3.40 ± 0.17	2.68 ± 0.39	2.84 ± 0.13
E4	2.22 ± 0.47	2.53 ± 0.89	2.21 ± 0.62	3.48 ± 0.18	2.21 ± 0.62
E5	2.38 ± 0.62	3.71 ± 0.77	3.0 ± 0.40	3.22 ± 0.30	3.11 ± 0.89
E6	2.75 ± 0.68	2.81 ± 0.60	3.20 ± 0.63	2.83 ± 0.64	2.67 ± 0.48
E7	3.54 ± 0.31	3.66 ± 0.69	2.80 ± 0.45	3.71 ± 0.64	3.91 ± 0.65
E8	2.12 ± 0.67	2.39 ± 0.29	3.30 ± 0.88	2.90 ± 0.74	2.30 ± 0.28
E9	2.33 ± 0.25	3.87 ± 0.50	2.60 ± 0.68	3.00 ± 0.23	3.06 ± 0.95
E10	2.83 ± 0.52	2.76 ± 0.54	3.30 ± 0.46	2.69 ± 60	3.37 ± 0.41
E11	2.18 ± 0.33	3.03 ± 0.32	2.80 ± 0.38	2.18 ± 0.33	2.26 ± 0.36
E12	3.37 ± 0.89	3.67 ± 0.73	3.54 ± 0.40	3.75 ± 0.68	3.49 ± 0.58
E13	2.75 ± 0.58	3.10 ± 0.43	2.90 ± 0.34	3.83 ± 0.41	2.79 ± 0.37
E14	3.02 ± 0.19	3.14 ± 0.15	3.60 ± 0.16	3.08 ± 0.10	2.60 ± 0.16
E. coli 1917 strain Nissle	6.60 ± 0.26	6.62 ± 0.40	6.28 ± 0.23	5.74 ± 0.58	5.28 ± 0.75
–	6.67 ± 0.11	6.45 ± 0.32	6.80 ± 0.50	6.28 ± 0.27	6.16 ± 0.50

[a] *C. botulinum* Type A, B, C, D and type E (10^4/ml) cultured anaerobically in a co-culture with *Enterococcus* spp. (10^4/ml) for 5 d. *C. botulinum* quantified using the most probable number (MPN) estimation method. Data expressed as reciprocal log_{10}. *Enterococcus* spp. prevent the growth of *C. botulinum* types A, B, C, D and E. *E. coli* 1917 strain Nissle has no effect on the growth of *C. botulinum* types A, B, C, D and E.

Inhibition of BotNT type B production by co-cultivation with E. faecalis and E. faecium
Even low numbers of E. *faecalis* (Fig. 1A) and E. *faecium* (Fig. 1B) inhibited BoNT production by all *C. botulinum* strains. Glyphosate itself also inhibited growth and BoNT expression of *C. botulinum* type B at relatively high concentrations (1 mg/mL).

Fig. 1. Influence of *Enterococcus faecalis* (A) and *E. faecium* (B) populations on neurotoxin production by 10^4/mL *C. botulinum* type B in a co-culture incubated for 5 days anaerobically. *C. botulinum* neurotoxin was determined by ELISA.



Influence of herbicides on bacterial growth and BotNT production
Roundup is more toxic to *C. botulinum* than to *E. faecalis*. The inhibitory effect of Roundup on the growth and toxin production of *C. botulinum* type B was more than 1 mg/mL (Table 3). Supplementation of the medium with 1 or 10 mg/mL glyphosate Roundup and glyphosate reduced the cell numbers of *C. botulinum* type B about 100 fold after 5 days of cultivation, respectively. The inhibitory concentrations of glyphosate to *C. botulinum* type B were 10-100 fold higher than the suppressed growth (0.1 mg/mL and 1 mg/mL) of *E. faecalis*.

Table 3: Influence of glyphosate and roundup on the growth of *C. botulinum* type B and E. *faecalis.*

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

[a] Glyphosate (N-Phosphonomethyl) glycine).
[b] Herbicide formulation (Roundup).
[c] *C. botulinum* type B (10^4/ml) cultured anaerobically in reinforced clostridial medium (RCM) containing different concentrations of glyphosate or herbicide formulation for 5 d. *C. botulinum* quantified using the most probable number (MPN) estimation method. Data expressed as reciprocal $\log_{10}$.
[d] *C. botulinum* type B quantified by ELISA.
[e] *E. faecalis* cultured aerobically in RCM containing different concentrations of glyphosate or herbicide formulation for 8 h and quantified on citrate-acid-tween-carbonate (CATC) agar. Data expressed as reciprocal $\log_{10}$.

Conclusion

In conclusion, the results suggest that ingestion of the herbicide glyphosate could be a significant predisposing factor that is associated with the increase in *C. botulinum* mediated diseases in cattle, currently being experienced on dairy farms due to loss of the antagonistic bacteria as enterococci from the GIT. This hypothesis needs to be confirmed by further tests and in vivo experiments.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case c) Relevance cannot be determined: Potential effects on gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus the relevance of the effects remained unclear. Moreover, the doses used in this study are not justified and are unrealistically high. Cultures are batch culture and it is unclear if conditions are to get values in the growing phase. The authors' hypothesis on the correlation between glyphosate exposure and the potential to increase in *C. botulinum* mediated cattle diseases need to be further verified.

No batch, purity or CAS No. of the test substance reported. No historical control data given.

The data on the formulation are not relevant for the glyphosate EU renewal (formulation used is not the representative formulation MON 52276). Furthermore, in the absence of a concurrent control for each to the component of the formulation, it is not possible to conclude whether the observed effects claimed to be secondary to exposure to glyphosate are due to glyphosate exposure or of one of the other components.

Assessment and conclusion by RMS:

In this study it is investigated if glyphosate or Roundup UltraMax could be a predisposing factor that is associated with the increase in *C. botulinum* mediated diseases in cattle by inhibiting *Enterococcus* spp. Which antagonises *C. botulinum*. The inhibitory effect of different *Enterococcus* spp. was studied by addition of different bacterial dilution to *C. botulinum* culture medium and additionally the inhibitory activity of glyphosate on *Enterococcus* and specifically on *enterococcus faecalis* was investigated. It was shown that *Enterococcus* isolated from faeces of cattle, horses and algae inhibited neurotoxin production by *C. botulinum*. At the lower concentration tested glyphosate and Roundup, for which the effect was stronger, inhibited growth of *E. faecalis*. At the higher concentrations tested glyphosate and Roundup also inhibited the growth of *C. botulinum*.
Since investigation of the gut microbiota is currently not part of the European assessment framework for pesticides, it is hard to assess the relevance of the findings. It is agreed with the conclusions by the applicant.

B.6.8.2.21. Public literature – Evidence by US autism epidemic initiated by acetaminophen is aggravated by oral antibiotic and now Roundup (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.8.2
Report author	Good P.
Report year	2018
Report title	Evidence by the U.S. autism epidemic initiated by acetaminophen (*Tylonel*) is aggravated by oral antibiotic amoxicillin/clavulanate (*Augmentin*) and now exponentially by herbicidade glyphosate (*Roundup*)
Document source	Clinical Nutrition ESPEN (2018), Vol. 23, pp. 171-183
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing	Not applicable

facilities	
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevance cannot be determined (EFSA GD Point 5.4.1 - category C)

2. Full summary of the study according to OECD format

This is a commentary with no original data. It involves a series of speculations.

Materials and methods

Not applicable. The paper is a commentary. Roundup herbicide was mentioned in the article but without any specific information.

Results

Not applicable. There are no original results. Just one author's narrative about various research that has been done with conclusions that do not stand up to reasonable scientific evidence criteria.

Conclusion

The U.S. autism epidemic has acetaminophen, amoxicillin and glyphosate among the causal factors.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. This paper contains no new data. It uses computer algorithms to make associations that are not proved. It claims that glyphosate impacts methionine and tryptophan and ignores that these amino acids are not only essential for the human diet but that microbially derived amino acids are only available via coprophagy.

Further points for clarification:
This paper is mainly speculation. It had no relevance to the ongoing glyphosate evaluation. Accepted scientific approaches to assess effects are not specified, thus relevance of the various works cited are unclear. This paper contains no new data. It uses computer algorithms to make associations that are not provable.

Assessment and conclusion by RMS:

This is not a research article, nor a review article. The article consists of quotes of other research/statements and commentary and seems to be an opinion paper.
The author speaks of an US autism epidemic from about the 1980s. This period coincides with the switch in advice for use of acetaminophen instead of aspirin in children. The author refers to an online parent survey that found children given acetaminophen for pain/fever of measles-mumps-rubella vaccine were far more likely to become autistic than children given ibuprofen. It is argued that acetaminophen depletes the liver's sulfate and glutathione thus impairing detoxification of other harmful agents.
The author also quotes other research that claims there might be a link between oral antibiotic amoxicillin/clavulanate and the so called autism epidemic: from 1981 this drug was prescribed and parents have reported regression into autism in their children. It is argued that amoxicillin/clavulanate eliminates normal gut flora resulting in overgrowth of pathogenic flora and that autistic regression between 12-18 month was commonly associated with GI symptoms.
Thirdly, glyphosate (Roundup) is mentioned in this article as causing aggravation of the autism epidemic. This link is made because the use of this herbicide has increased since the last decades and it is used for foods that are a large part of current diets in the US. It is speculated that glyphosate might influence gut bacteria.
The article claims the following: *The pathologies seen in autism are explained by depletion of placental/postnatal estrogens by 1) acetaminophen depleting sulphate and glutathione thus DEAS (and glutamine), 2) amoxicillin/clavulanate killing and glyphosate inhibiting gut flora that synthesize methionine – precursor of sulfate, glutathione and DHEAS; and 3) glyphosate (and heavy metals) inhibiting aromatase, thus conversion of androgens to estrogens.*

This article consists mainly of speculation and no details on the articles cited is included, therefore, the research behind the claims is unclear. The article is considered to be unreliable.

B.6.8.2.22. Public literature – Sex-dependent impact of Roundup on rat gut microbiome (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.8.2
Report author	Lozano V.L. *et al*.
Report year	2018
Report title	Sex-dependent impact of Roundup on the rat gut microbiome
Document source	Toxicology Reports (2018), Vol. 5, pp. 96-107
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevance cannot be determined (EFSA GD Point 5.4.1 - category C)

2. Full summary of the study according to OECD format

Human gut microbiome is inhabited by 10^{13}-10^{14} bacteria, more or less the same order as the number of human cells. A growing body of research indicates that dysbiosis of the gut microbiota is implicated in a wide range of clinical conditions, some of which develop local in intestine or some others developing in distant organs being more surprising, such as diabetes, obesity, asthma, liver and cardiovascular diseases, or even autism spectrum disorder. A number of studies have indicated that changes in dietary patterns, as well as the presence of toxic food contaminants, can modulate the composition and the activity of the gut microbiome. Thereby the effect of glyphosate-based herbicides in the formulation Roundup was tested.

During the study, faeces samples were collected from rats, which were part of a long-term study (673 days) in which 3 doses of Roundup (0.1 ppb, 400 ppm and 5000 ppm) had been administrated to 3 animals/sex and dose in tap water *ad libitum*.

In this study, a sex-dependent dysbiosis in females was observed, associated with corresponding effects in the affected females at an environmental concentration of Roundup (0.1 ppb) and other two concentrations (400 ppm and 5000 ppm) and thus warrants further investigation.

Materials and methods

Test Material:	Roundup Grand Travaux Plus® (450 g/L glyphosate)
Origin:	Monsanto, Belgium
Lot/Batch number:	Not reported
Purity:	Not reported
CAS#:	Not reported
approval	2020448
Vehicle:	Tap water
Test Animals:	
Species	Rat
Strain	Sprague-Dawley
Sex	Male and female
Age/weight on arrival	Not reported
Source	Not reported
Housing	Single housing; only two males treated with 0.1 ppb Roundup were housed together
Acclimatisation period	Not reported
Diet	Not reported

Sample selection
The glyphosate concentration in drinking water, as well as the glyphosate stability, during the 7-day period between two preparations of the test, was confirmed by HPLC-MS/MS (corresponding to 50 ng/L, 0.1 g/L and 2.25 g/L of glyphosate respectively).

The faeces samples (n = 24) were collected after 673 days of Roundup administration. They were kept at -80°C. Samples were selected from independent cages as recommended to avoid pre-clustering microbiota.

16S sequencing analysis
Twenty-four faecal samples were analysed by high-throughput sequencing IonTorrent in Ad Gene laboratory (Thury-Harcout, France) according to the manufacturer's instructions.

Samples were homogenized, and 200 mg were treated with Nucleospin Tissue (Macherey Nagel, Hoerdt, France) for DNA extraction. DNA quantity was measured by spectrophotometer (Biophotometer, Eppendorf, Montesson, France).

A total of 7 hypervariable zones (V2, V4, V8, V3, V6, V7 and V9) of 16S rRNA gene were amplified with the Ion 16 Metagenomics Kit (Life Technologies, Saint-Aubin, France). The amplification was made with two sets of primers (V2-4-8 and V3-6.7-9). The results were controlled by capillary electrophoresis analyses using the QIAxcel Advanced System (Qiagen, Courtaboeuf, France). Amplified DNAs were purified with the MinElute Purification Kit (Qiagen).

Preparation of libraries was performed with the Ion Plus Fragment Library Kit (Life Technologies), and 4 samples were treated in each sequencing run using Ion Xpress Barcodes Adapters 1-16 and Ion Xpress Barcode Adapters 17-32 (Life Technologies). All libraries correspond to DNA fragments from 200 bp to 400 bp. Emulsion PCR was made with the Ion PGM Template OT2 400 Kit in OneTouch2, the enrichment of balls was realized in OneTouchES. Quality control was carried out with the Ion Sphere™ Quality Control Kit in Qubit 2.0 fluorimeter. Sequencing was undertaken in the 316v2 microchip with Ion PGM Hi-Q Sequencing Kit on the ION PGM sequencer.

All the corresponding raw data has been posted on the ENA database with the accession number ERP104935 (PRJEB23198).

Repetitive sequence-based PCR (rep-PCR)
A total of 72 faecal samples were analysed by (GTG)5-PCR. DNA was extracted and DNA samples were further diluted 10 times to prevent any risk of saturation of the polymerase.

Capillary electrophoresis analysis was performed on the QIAxcel Advanced System (Qiagen, Courtaboeuf, France) according to the manufacturer's recommendations. For each electrophoresis process, a QIAxcel DNA High Resolution Cartridge, an Alignment Marker 50 bp/5000 bp, a size marker 250 bp-4000 bp, and the OM 500 method were used.

The electrophoresis analyses were performed using the QIAxcel Screengel Software v1.2.0 (Qiagen), and the DNA fingerprint gel images were analysed with the GelJ v1_3 software. Dendrograms were generated using similarity method (Pearson's correlation), linkage (UPGMA) and tolerance of 2%.

Bioinformatic analysis
The analysis of 16S sequencing data was performed with the Torrent Suite Software (v4.4) and ION Reporter (v4.4). The bioinformatics workflow corresponded to Metagenomics 16S beta. The following parameters were used: Curated MicroSEQ® 16S Reference Library V2013.1 (Data Bank), size minimum of reads 150 bp, percentage of alignments for identification 90%, minimal read abundance for validation 10, cut-off Gender 97% minimal alignment, cut-off species 99% minimal alignment, slash-call was defined as 0.8%.

The data were expressed as percentages of phylum and families from a taxonomical consensus between the 7 hypervariable regions. 16S sequencing data were then analysed by multivariate analysis with the SIMCA-P (V13) software (Sartorius Stedim Data Analytics AB Malmö, Sweden). Variables (phylum and families) were mean-centred but not scaled (all the variables are expressed in the same unit) prior to analysis.

A first analysis was carried out by Principal Component Analysis (PCA). A second analysis was carried out by using an Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA). The objective of discriminant analysis is to find a model that separates classes of observations on the basis of their X variables (16S sequencing data) and the Y matrix (class of each observation).

Microbial strains and culture conditions

First, a selection protocol on faecal samples from control (n = 6) and Roundup 5000 ppm (n = 6) treated rats using media prepared according to published methodology was performed. Briefly, dilutions of faecal samples (aiming to obtain 30-300 CFU per Petri dish) were incubated in selective agar medium in triplicate (Table 1) and counted (252 cultures). Control bacterial strains were incubated in liquid media (RCM broth) with different concentrations of Roundup (0.1 ppb, 400 ppm and 5000 ppm) in triplicate. After 24 h of treatment, samples were diluted to appropriate factor to allow counting, cultured (24 h) in selective agar media, and afterwards, all colonies were counted.

Table 1. Media and conditions for strains isolation and culture.

Selection	Media	Conditions
Agar Plates		
Total Aerobes	Clostridia Reinforced Agar (Biokar)	72 h Aerobically, 37 °C
Total Anaerobes	Clostridia Reinforced Agar (Biokar)	72 h Anaerobically (AnaeroGen 2,5 L, Sigma-Aldrich), 37 °C
Bifidobacteria (Muñoa and Pares, 1988)	51 g Clostridia Reinforced Agar (Biokar), 0,02 g nalidixic acid (Sigma-Aldrich), 0,0085 g polymyxin B sulfate (Biokar), 0,05 g kanamycin sulfate (Sigma), 0,025 iodoacetic acid (Sigma), 2,3,5-triphenyltetrazolium chloride (TTC, Biokar)	72 h Anaerobically (AnaeroGen 2,5 L, Sigma-Aldrich), 37 °C
Lactobacillus	ROGOSA (Biokar), Acetic Acid (Sigma-Aldrich)	72 h Anaerobically (AnaeroGen 2,5 L, Sigma-Aldrich), 37 °C
Enterococci	Slanetz et Bartley (Biokar), 2,3,5-triphenyltetrazolium chloride (TTC, Biokar)	48 h Aerobically, 37 °C
Coliforms	MacConkey Agar 3, (Oxoid)	24 h Aerobically, 37 °C
Clostridia	Clostridia Reinforced Agar (Biokar), 20 mg/L polymyxin B sulfate (Biokar)	72 h Anaerobically (AnaeroGen 2,5 L, Sigma-Aldrich), 37 °C
Broth (liquid media)		
General	Reinforced Clostridial Medium (Biokar)	24 h Aerobically, 37 °C
Lactobacillus	MRS Broth (Biokar)	24 h Aerobically, 37 °C

To study the dose-response relationship of Roundup toxic effects by exposing the different strains isolated from rat faeces (control group) to Roundup for 24 h. Roundup and Glyphosate have been adjusted with NaOH to pH = 7 to avoid acidity impacts and filtered (0.22 µm) to eliminate possible contamination. After 24 h of exposure, the absorbance (600 nm) was measured, and 50% of growth inhibition (MIC50) intervals were estimated. The inhibition rate was confirmed on agar plates with the same concentration of Roundup.

Characterization of a Roundup-tolerant or resistant bacterial strain

One strain isolated on RCM agar plate (Biokar Diagnostics, France) had a particular phenotype when cultivated with 5000 ppm of Roundup. A colony was pelleted and seeded with and without 5000 ppm of Roundup in 10 mL of liquid RCM. After 72 h at 37 °C, cells were centrifuged, washed in physiological water, and concentrated to be analysed by Fourier Transform Infrared spectroscopy (FT-IR). IR spectroscopy is a simple and cheap tool able to give rapid global information about the physiological status of microorganisms, as IR spectra reflect the global chemical composition of the sample. It can provide information on existing taxonomic differences, or on chemical changes owing to stressful environments. Registered spectra were analysed with OPUS 6.5 (Bruker) software, on spectral windows 3100–2800 + 1500-1350 cm^{-1}, 1800-1500 cm^{-1} and 1200-900 cm^{-1} for studying fatty acids, proteins and polysaccharides, respectively. Spectra comparisons were performed by hierarchical cluster analysis (HCA) using vector normalization of first derivative and Ward́s algorithm. Taxonomical determination was performed by the API 20E phenotypic assay (bioMérieux SA, France) and Sanger sequencing of 16S RNA gene as follows.

DNA was extracted with the Kit EZ1 DNA Tissue (Qiagen). A total of 200 µL of bacterial strain was centrifuged for 5 min at 8000 rpm. The supernatant was removed and 200 µL of G2 buffer was added. The suspension was vortexed and placed into an EZ1 2 mL microtube before extraction was done with the automatic extractor EZ1 Advanced system (Qiagen). A total of 200 µL of DNA was obtained. Amplification was made with primers 16S-Bact-8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 16S-Bact-1510R (5′-GGTTACCTTGTTACGACTT-3′) for 16S DNA and with primers EPSPS-P1-F (5′-CGGGATCCATGGAATCCCTGACGTTACAA-3′) and EPSPS-P2-R (5′-GCGGATCCTCAGGCTGCCTGGCTA ATC-3′) for EPSPS gene. The cycling conditions were as follows. A first denaturation was performed for 5 min at 95 °C, followed by 30 cycles made of a denaturation step of 30 s at 95 °C, an annealing step of 30 s at 60 °C and an elongation step of 90 s at 72 °C. Amplification results were controlled by capillary electrophoresis analyses performed on the QIAxcel Advanced System (Qiagen, Courtaboeuf, France). Amplified DNAs were purified with MinElute Purification Kit (Qiagen) following manufacturer protocol. Purified amplicons were sent to Eurofins Genomics (Ebersberg, Germany) for Sanger sequencing.

Statistical analysis

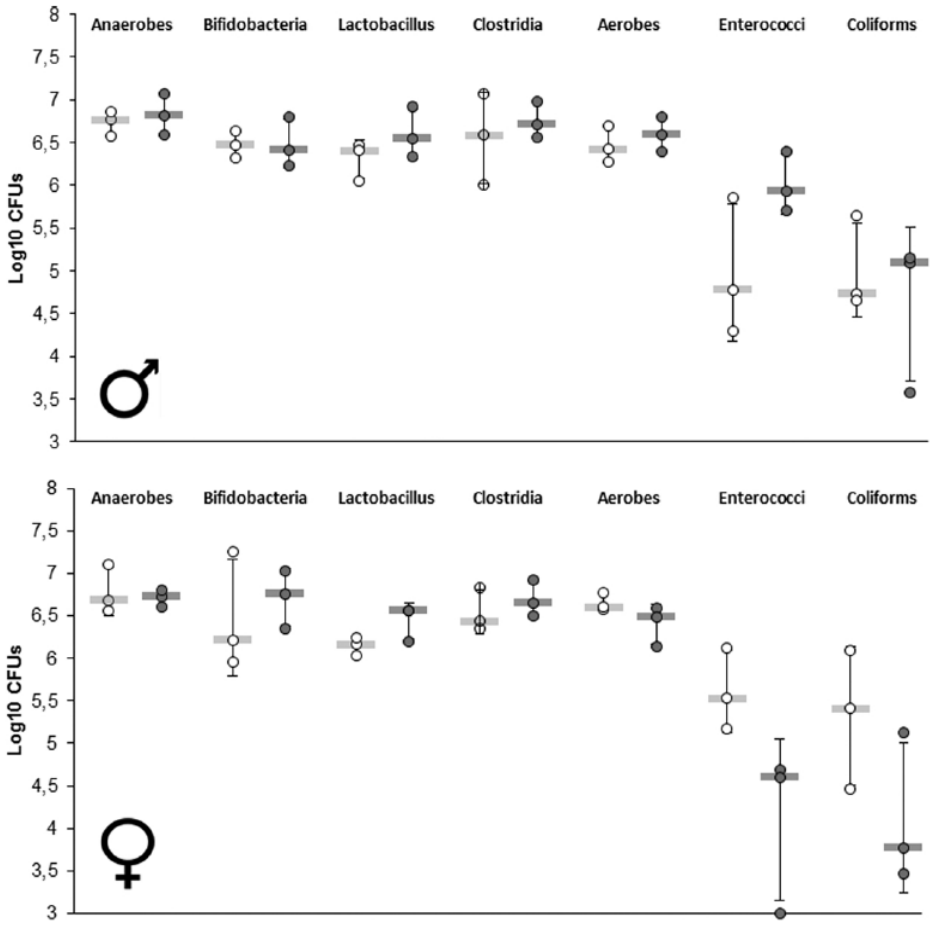
Statistical differences were determined by a non-parametric Kruskal-Wallis test, using InfoStat version 2012 software (InfoStat Group, Cordoba, Argentina).

Results

Effects of Roundup towards microbiome genomic diversity

Significant differences between treated females and all other groups of rats (control males, control females and treated males) could be shown. These differences mostly consisted of an increase in the *Bacteroidetes* family S24-7 and a decrease *Lactobacillaceae* in 8 out of the 9 females treated with different doses of Roundup. In order to control possible distortion of DNA extraction and/or amplification, repetitive sequence-based PCR (rep-PCR) profiles were realized, using the same batch of extraction and 2 additional extractions. The three profiles are slightly discordant. The results highlight significant differences between treated females and all other groups of rats (control males, control, females and treated males). These differences mostly consisted of an increased in the *Bacteroidetes* family S24-7 and a decrease *Lactobacillaceae* in 8 out of the 9 females treated with different doses of Roundup. In order to control possible distortion of DNA, extraction and/or amplification repetitive sequence-based PCR (rep-PCR) profiles were realized, using the same batch of extraction and 2 additional extractions. The three profiles are slightly discordant but they all confirm the 16S sequencing results in 8 of the 9 treated females, which clearly separate from controls (males and females) and treated males (Fig. 1).

Figure 1. Determination of microbiome composition by traditional culture method. Results of colony-forming units numerations of controls (white) and Roundup 5000 ppm treatment (grey) samples of rat faeces (males n = 3, females n = 3), to compare traditional culture methods and 16S high-throughput sequencing.



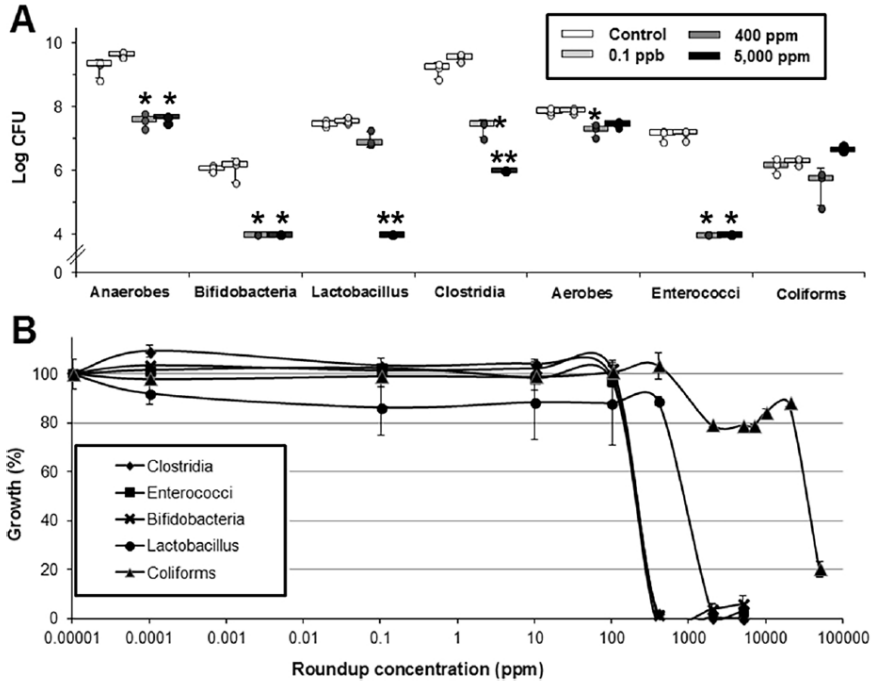
Roundup chronic effects in vivo: microbiome cultivable biodiversity

In order to be able to compare the microbiome of 3 males and 3 females treated with 5000 ppm of Roundup and their relative control the total anaerobes, total aerobes, *Clostridia*, *Bifidobacteria*, *Lactobacilli, Enterococci* and *Coliforms* in faeces were measured and compared. The results did not reflect the trends observed in the 16S sequencing analysis. A very strong method deviation, especially in *Enterococci* and *Coliform* population was observed. This making it very difficult to observe possible treatment-related effects.

Roundup short-term effects in vitro

A significant growth inhibition at the two highest concentrations (400 and 5000 ppm) of total anaerobes population, *Bifidobacteria*, *Clostridia*, and *Enterococci* was observed (Fig. 2). *Lactobacilli* were less sensitive; their growth was not altered at 400 ppm administered Roundup. Coliforms were not sensitive to any of the Roundup concentrations tested. This is confirmed by the study of the effects of increasing concentrations on isolated gastrointestinal strains. It suggests that gut microbiome disturbances provoked by Roundup exposure can be due to a direct selective bactericidal action, although the MIC50 were very high in comparison to the Roundup concentration administered in vivo.

Figure 2: Differential impact of Roundup on bacteria inhabiting the gastrointestinal tract of rats.
(A) Impact on community after 24 h of treatment by Roundup. The median (n = 3) and SD are shown. *p <0.05, **p <0.01.
(B) Dose-response of Roundup inhibitory effects on the growth of isolated gastrointestinal strains from rat faeces.



Conclusion

The high-throughput 16S sequencing analysis revealed that Roundup exposure via drinking water caused sex-specific alterations of the rat gut microbiome, reflected by an increase in the *Bacteroidetes* family S24-7 and a decrease in *Lactobacillaceae* in females. Roundup had a direct selective bactericidal action on isolated gastrointestinal strains, which could explain the effects observed *in vivo*. A dose-dependency concerning the composition of the microbiome could not be observed. However, the gut microbiome has been shown to be resilient, while it should be kept in mind that non-cultivatable bacterial communities of the microbiome were not investigated. The toxicity of Roundup and the effect on gut microbiome could be either due to Glyphosate or its formulants, or to a synergistic effect of all components. In addition, the age and health conditions of the rats were not mentioned and are fundamental factors for the composition and effectivity of the microbiome, as well as the endocrine system of male and females defining the microbiome were not taken into account. In addition, neither the amount of captured test substance by the rat was determined, nor the blood plasma concentration as proof of intake and internal distribution in the animal was confirmed.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case c) Relevance cannot be determined: Potential effects on gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus the relevance of the effects remained unclear. Moreover, the doses used in this study are not justified and are unrealistically high. Cultures are batch culture and it is unclear if conditions are to get values in the growing phase. Comparisons between glyphosate and Roundup are completely different so they cannot be compared.

The data on the formulation are not relevant for the glyphosate EU renewal (formulation used is not the representative formulation MON 52276). Furthermore, in the absence of a concurrent control for each to the component of the formulation, it is not possible to conclude whether the observed effects claimed to be secondary to exposure to glyphosate are due to glyphosate exposure or of one of the other components.

This study has a number of issues related to design: Rats are at the end of their life when faeces were sampled. It is not clear of faeces were sampled pre- or post mortem. Results are confounded by advanced age or even tumour status of these rats, predominantly mammary. The smaller than expected number of phyla may be related to the age of the rats. Short-term responses are not surprising: cells in direct contact with a substance in a test tube (liquid medium) will respond differently than cells exposed to that same substance within their natural environment. So in vitro data usually show cells have a greater sensitivity to the substance than in vivo data. And within the intestinal environment, there is much to dilute, diminish or mask the substance's effect. This diminished effect in vivo has been documented repeatedly for a large number of test substances. Effects of surfactants in the used product were not taken into account. It is difficult to conclude on the toxicological relevance of this study as no information was provided on clinical signs, body weight, food consumption, no blood clinical analysis or urin alysis was reported.

No batch, purity, or CAS No. of the test substance reported. Strain and source of test animals not specified. Diet, acclimatisation period, and housing of animals not described. Substance intake not calculated. No positive control used. No historical control data given.

Assessment and conclusion by RMS:

In this study investigation into gut microbiome were performed on rats exposed long-term to a Roundup formulation at three different dose levels via drinking water. Following high-throughput 16S sequencing analysis, differences were seen in treated females compared to the other groups (control females, control and treated males); dose dependency was not observed. In addition, short-term effects on microbiota by cultivation of faecal bacteria was studied where no effect was found.

Gut microbiome is not part of the data requirements. In June 2020, EFSA published an editorial on exploring the need to include microbiomes into EFSA's scientific assessments (EFSA Journal 2020;18(6):e18061). In this editorial EFSA highlights that gut microbiome research is expected to play a relevant role in regulatory science and that further research is needed to enhance the understanding of the toxicological significance of microbiome-mediated metabolism of chemicals. Sequencing based tools and multi-omic approaches still need considerable work to refine and integrate them into study design as well as analytical standardisation.

This study is considered to be supplemental information only.
The study is carried out with a formulation of glyphosate, thus side effects caused by co-formulants cannot be excluded. The study was not conducted according to any international guideline, no GLP status, the test substance is not sufficiently characterised (particularly, purity and batch not specified) and no justification for the doses is given, details on animals (e.g. source, weight) were not given, dosing was conducted via drinking water with no data on intake given in the article, therefore exposure is unknown. Limited to no information on systemic toxicity in the animals (e.g. clinical signs, body weight, food consumption, organ weight and histopathology) was given, low number of animals used, individual data missing, no historical control data and positive control missing.
Based on the aforementioned limitations, the study is considered to be unreliable.

B.6.8.2.23. Public literature – The Ramazzine Institute 13-week pilot study: effects on microbiome (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.8.2
Report author	Mao Q. *et al*.
Report year	2018
Report title	The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome
Document source	Environmental Health (2018), Vol. 17, Issue 1, pp. 50
Guidelines followed in study	Legislative Decree No. 26 implementing Directive 2010/63/EU on the protection of animals used for scientific purposes.
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevance cannot be determined (EFSA GD Point 5.4.1 - category C)

3. Full summary of the study according to OECD format

The present pilot study examines whether exposure to glyphosate-based herbicides (GBHs) at doses of glyphosate considered to be "safe" (the US Acceptable Daily Intake - ADI - of 1.75 mg/kg bw/day), starting from in utero, may modify the composition of the gut microbiome in Sprague Dawley (SD) rats.

Glyphosate alone and Roundup, a commercial brand of GBHs, were administered in drinking water at doses comparable to the US glyphosate ADI (1.75 mg/kg bw/day) to F0 dams starting from the gestational day (GD) 6 up to postnatal day (PND) 125. Animal faeces were collected at multiple time points from both F0 dams and F1 pups. The gut microbiota of 433 faecal samples were profiled at V3-V4 region of 16S ribosomal RNA gene, and further taxonomically assigned and assessed for diversity analysis. The effect of exposure on overall microbiome diversity was determined using PERMANOVA and on individual taxa by LEfSe analysis.

Microbiome profiling revealed that low-dose exposure to Roundup and glyphosate resulted in significant and distinctive changes in overall bacterial composition in F1 pups only. Specifically, at PND31, corresponding to pre-pubertal age in humans, relative abundance for *Bacteriodetes (Prevotella)* was increased while the *Firmicutes* (*Lactobacillus*) was reduced in both Roundup and glyphosate exposed F1 pups compared to controls.

This study provides initial evidence that exposures to commonly used GBHs, at doses considered safe, are capable of modifying the gut microbiota in early development, particularly before the onset of puberty. These findings warrant future studies on potential health effects of GBHs in early development such as childhood.

Materials and methods

Test Material:	Glyphosate (I)
	Roundup (II)
Origin:	Not reported
Lot/Batch number:	Not reported
Purity:	Not reported
CAS#:	Not reported
Vehicle:	Drinking water
Positive control:	None
Test Animals:	

Species	Rat
Strain	Sprague Dawley (SD)
Sex	male
Age/weight on arrival	Not reported
Source	CMCRC/RI animal breeding facility
Housing	Parental animals: Individually in polycarbonate cages
	Offspring: 3/sex
Acclimatisation period	Not reported
Diet	Not reported
Environmental conditions:	
Temperature	22 ± ° C
Humidity	50 ± 20 %
Light/dark cycle	12 hours light and 12 hours dark

Experimental model
The CMCRC/RI animal breeding facility was the supplier for the Sprague-Dawley (SD) rats. Female rats were individually co-housed with a single male in Polycarbonate cages until mating occurred. After mating, matched females were housed separately during gestation and delivery. Newborns were housed with their mothers until weaning. Weaned offspring were co-housed, by sex and treatment group, not more than 3 per each cage.

Analysis of chemical characteristics (pH, ashes, dry weight, specific weight) and possible contamination (metals, aflatoxin, polychlorobiphenyls, organophosphorus and organochlorine pesticides) of the bedding was performed by CONSULAB Laboratories (Treviso, Italy).

Experimental protocol
Two groups of SD rat dams (in total 24) and relative pups were treated with either glyphosate or Roundup diluted in drinking water at the glyphosate concentration of 1.75 mg/kg bw/day. The F0 female animals received the treatment from gestation day (GD) 6 to the end of lactation. The offspring (F1) were retained in their litter with the respective dams until weaning. After weaning, animals from the F1 generation were randomly distributed in two cohorts: animals belonging to the 6-week cohort were sacrificed at PND 73± 2, i.e. 6 weeks after weaning, animals belonging to the 13-week cohort were sacrificed at PND 125 ± 2, i.e. 13 weeks after weaning. After weaning, the F1 generation was treated with glyphosate or Roundup via drinking water until sacrifice.

The timeline of the experimental animal treatment and faecal sample collection is shown in Fig. 1. As illustrated, rat faecal samples were individually collected from all animals of the F0 generation (8 dams) from each group before mating, at GD 5 (before the starting of the treatment), GD 13, lactation day (LD) 7 and LD 14.

Faecal samples were also collected from 108 F1 pups, 18 males and 18 females from each group during lactation at PND 7 and PND 14 (corresponding to LD 7 and 14 for dams), before the achievement of puberty at PND 31, after puberty at PND 57 and in adulthood at PND 125. Due to technical difficulty to identify faecal samples from individual pups during lactation, only pooled samples at PND 7 and PND 14 were collected for each cage from the whole litter, not distinguished by gender. After weaning, faecal samples from each pup were individually collected. About 2–3 droppings, collected directly from the anus of each animal, were preserved in cryovials on an ice bed then stored at − 20 °C until shipment on dry ice.

Bacterial 16S PCR and sequencing
Rat faecal DNA was extracted using the QIAamp PowerFecal DNA Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Total DNA concentration was determined by Qubit 2.0 Fluorometer (Life Technologies, Norwalk, CT). The phylogenetically informative V3–V4 region of 16S rRNA gene was amplified using universal primer 347F/803R with dual-barcoding approach. The integrity of the 16S PCR amplicons was verified by agarose gel electrophoresis. The resulting ~ 460-bp sized amplicons were pooled and then sequenced with the Illumina MiSeq 2 × 250 paired-end sequencing platform at OCS genome technology center of New York University Langone Medical Center.

16S data analysis
The sequencing data were merged and filtered to remove the merged reads with a length of < 400 bp or the

quality score of < Q30 at more than 1% of bases. Sequentially, all filtered high-quality reads were split by dual-barcode and trimmed of primer regions using a self-defined bash script to integrate several sequencing processing commands from fastx, QIIME, and seqtk. Duplicated measurements of four sample were processed and sequenced using different barcodes to test the sequencing reproducibility. Five blank samples were also sequenced and referenced to filter the possible environmental contamination during the sample procession. The split high-quality reads were further processed by QIIME 1.9.0. The command *pick_open_reference_otus.py* with the defaulted green_ gene 97_otus reference sequences was used to cluster of > 97% similar sequencing reads as an OTU using uclust. Representative sequences for each OTU were aligned using PyNAST and build the phylogenetic tree. Finally, the QIIME generated biom-formatted OTU table contains the taxonomic information and absolute counts for each identified taxon in each sample.

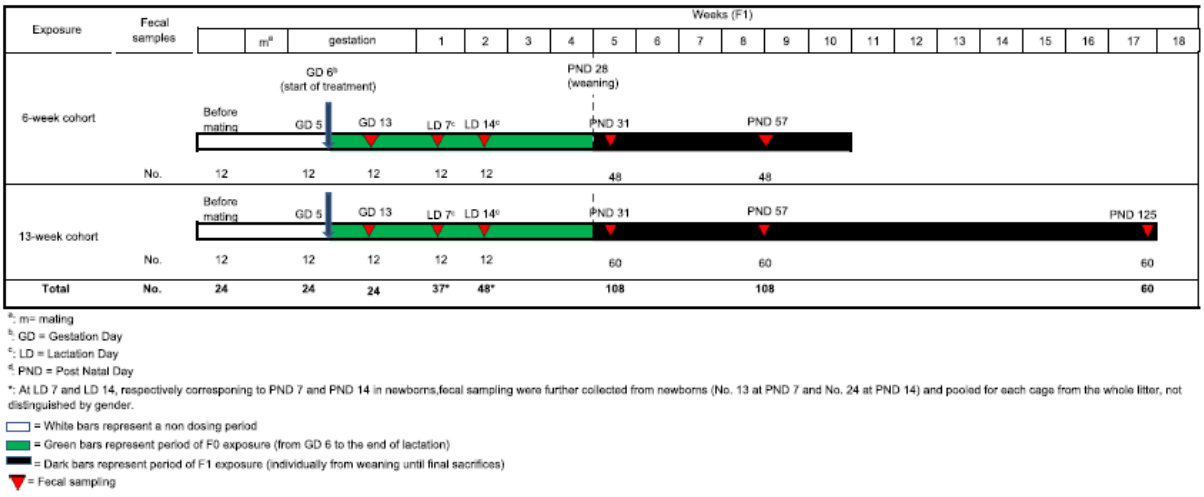
The diversity within each microbial community, so-called alpha-diversity, was calculated using the Shannon Index as metric and represented the measure of the diversity at the family and genus level. The overall microbiome dissimilarities among all samples were accessed using the weighted UniFrac distance matrices. Non-metric multiple dimensional scaling (NMDS) was used to visualize the dissimilarities. The permutational multivariate analysis of variance PERMANOVA test, with the maximum number of permutations = 999, was performed to assess the significance of the overall microbiome differences between groups by collection time points and treatment. The PERMANOVA procedure using the [Adonis] function of the R package vegan 2.0–5 partitions the distance matrix among sources of variation, fits linear models to distance matrices and uses a permutation test with pseudo-F ratios to obtain the p values. Using the LEfSe method, the microbiome features significantly associated with time of collection and treatments at various taxonomic ranks were further selected.

Results

No unexpected clinical signs or symptoms were observed in the experimental animals during the in vivo phase. In particular, no sign of changes in maternal behaviour during lactation (nesting and nursing) were observed during the experiment. There was no clinical evidence of alterations in activity or behaviour in pups. Body weight, water and feed consumption both in dams and pups were no different across the groups. Litter sizes were fully comparable among groups.

The total DNAs were extracted from 433 SD rat faecal samples (see Fig. 1).

Fig. 1 Timeline of the experimental animal treatment and faecal sample collection.



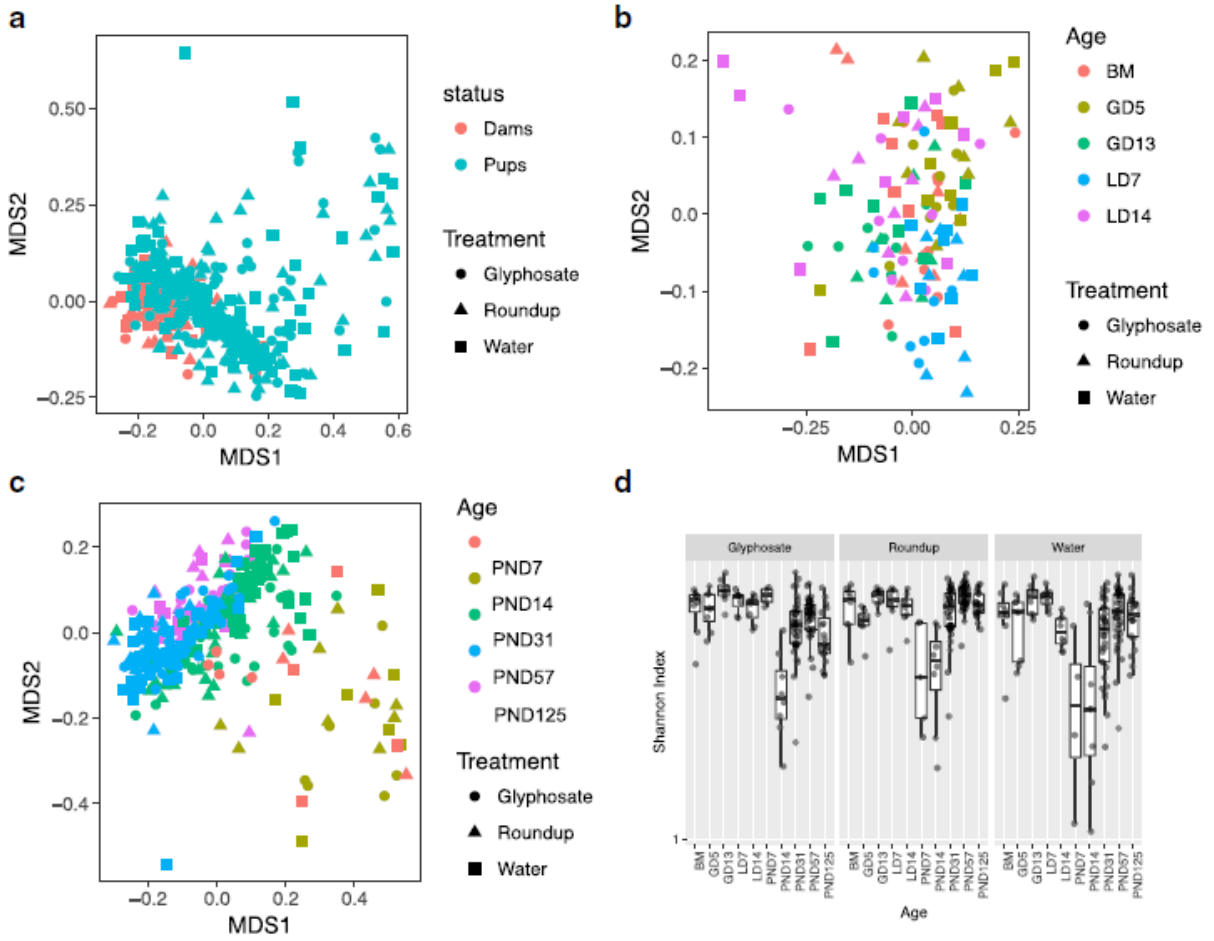
[a]: m= mating
[b]: GD = Gestation Day
[c]: LD = Lactation Day
[d]: PND = Post Natal Day
*: At LD 7 and LD 14, respectively corresponing to PND 7 and PND 14 in newborns,fecal sampling were further collected from newborns (No. 13 at PND 7 and No. 24 at PND 14) and pooled for each cage from the whole litter, not distinguished by gender.
= White bars represent a non dosing period
= Green bars represent period of F0 exposure (from GD 6 to the end of lactation)
= Dark bars represent period of F1 exposure (individually from weaning until final sacrifices)
= Fecal sampling

Faecal samples of pups at PND 7 and PND 14 showed significant low DNA yields. Further, microbiome survey on 433 SD rat faecal samples was performed, and 5 water blanks using bacterial 16S sequencing. Approximately 2 million high-quality reads were obtained. The number of reads was not significantly different by exposure type.

The overall microbiome dissimilarity was visualized by non-parametric multidimensional scaling (NMDS) plot of all samples (Fig. 2a), dams only (Fig. 2b) and pups only (Fig. 2c). The early postnatal samples at PND 7 and PND 14 were found to be far apart from the dams at LD 7 and LD 14 while the later postnatal samples at PND

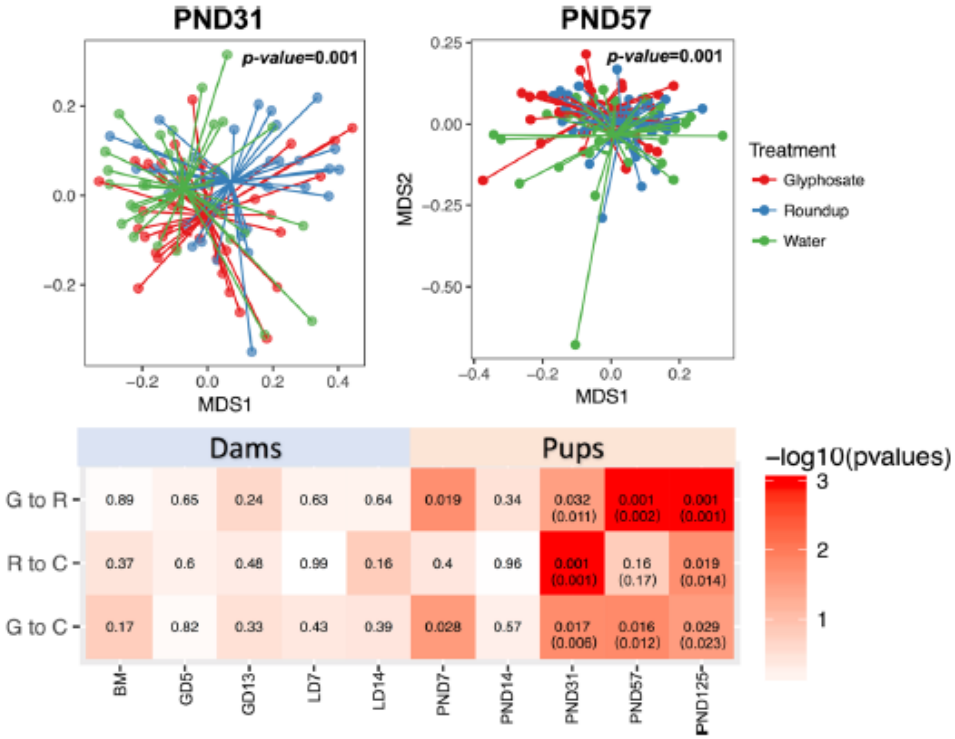
31, PND 57 and PND 125 were clustering with the dams (Fig. 2a). The mean and variance of the within-community diversity (α diversity) measured by Shannon index showed that the samples from dams possessed higher, while early postnatal samples from pups showed lower α diversity (Fig. 2d). Student t-test showed significantly increased α diversity from PND 14 to PND 31 (p-value < 0.05 for all treatment groups) but no differences between samples at the same age but different treatment group.

Fig. 2 The overall microbiome diversity. a, b, and c are non-metric dimensional scaling (NMDS) plots visualize the overall microbiome dissimilarities (beta-diversity) between individual rat across time. a) All samples from SD dams (pink) and pups (green) of three treatment groups; b) All samples from SD dam rats only. Colours indicate sample collection timepoint. BM: before mating; GD 5: gestation day 5; GD 13: gestation day 13; LD 7: lactation day 7; and LD 14: lactation day 14. c) All samples from SD pup rats only. Colours indicate sample collection timepoint. PND 7 to PND 125: postnatal day 7 to postnatal day 125. d) Box plots show the mean and variance of the within-community diversity (alpha-diversity) measured by Shannon index in three treatment groups across all time of collections.



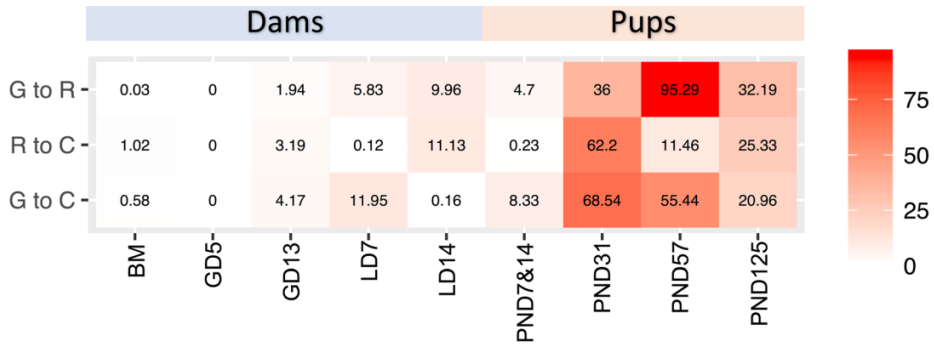
We compared the overall microbiome changes by treatment at different age groups from pups and dams. Nonmetric multidimensional scaling (NMDS) plots visualized the overall microbiome dissimilarities by treatment at PND 31 and 57 (Fig. 3a). The test results (p-values shown in Fig. 3b) showed that the overall microbiome was significantly altered by both Roundup and glyphosate treatment compared to controls. Similarly, there were significant differences in microbiota between Roundup and glyphosate exposed F1 pups. Further, the overall microbiome was significantly different by sex at PND 125 (p-value = 0.028, 0.007 and 0.013 by PER-MANOVA test for Glyphosate, Roundup and control group, respectively). To adjust for the sex effect, an additional multivariable PERMANOVA test with both treatment and sex as predictive variables was performed. The test results were consistent (Fig. 3b). However, none of the F0 dam groups showed significant differences in overall microbiota diversity.

Fig. 3 The effect of glyphosate exposure on overall microbiome diversity. a) NMDS plots visualize the overall microbiome dissimilarities (beta-diversity) between individual rat of three treatments at PND 31 and PND 57. b) PERMANOVA test is performed to test the significance among all three treatments (displayed in NMDS plots) and between two treatments (values are listed in tables). The p-values in parenthesis were adjusted for genders. G: glyphosate; R: Roundup; C: control water.



The linear discriminant analysis effect size (*LEfSe*) analysis was performed using 16S sequencing data from rat faecal samples in order to select particular discriminative features of the glyphosate exposure. Consistently with the overall microbiome changes by exposure at different age groups (Fig. 3), several significant differential taxa features associated with exposure were found. In particular, at PND 31, the results showed that the microbiota of both glyphosate and Roundup exposed pups had a significantly higher prevalence of *Prevotella genus (Bacteroidetes phylum)* and *Mucispirillum genus (Deferribacteres phylum)* and lower prevalence of *Lactobacillus genus (Firmicutes phylum)* and *Aggregatibacter genus (Proteobacteria phylum)*. However, some of the selected features were treatment specific. For instance, among the most significant features with LDA score > 3.0 and *p*-value< 0.05, increased *Blautia genus (Firmicutes phylum)* and decreased *Streptococcus genus (Firmicutes phylum)* and *Rothia genus (Actinobacteria phylum)* were found only in glyphosate exposed PND 31 pups, but not in Roundup exposed samples. In contrast, increased *Parabacteroides genus (Bacteroidetes phylum)* and *Veillonella genus (Firmicutes phylum)* were only found in Roundup exposed pups, but not in glyphosate exposed samples at PND 31. Between two exposures, Roundup exposed pups showed increased *Clostridia class (Firmicutes phylum)*, in particular, *Blautia* genus and *Actinobacteria* class (*Actinobacteria* phylum), in particular, *Rothia* and *Bifidobacterium* genera at PND 31. Furthermore, the treatment associated taxa features were not consistent at different postnatal time points. Many features selected at PND 31 did not appear at PND 57, suggesting that early-life microbiota was less stable the effect on gut microbiota was continuous. When counting the total abundance % of the significant differential taxa by treatments, the pups showed much higher impact by exposure than the dams (Fig. 4).

Fig. 4 The table lists the overall abundance of the significant differential taxa between treatments across time.



Conclusion

The results revealed that both glyphosate and glyphosate formulated Roundup, at doses admitted in humans, including children and pregnant women, significantly altered the microbiota diversity and resulted in prominent changes at multiple taxa in exposed pups. However, those effects on microbiota were not significant in the adult dams. The data suggests that the prepubertal age microbiota is more sensitive to GBH exposure compared to the adult microbiota, therefore the postnatal age is likely a "window of susceptibility" for GBHs to modulate the gut microbiome.

Furthermore, the results showed that the overall microbiome diversity and composition were significantly different between Roundup and glyphosate, suggesting possible synergistic effects of the mixed formulation on gut microbiota.

This pilot study provides initial evidence that maternal exposure to commonly used GBHs, at doses currently considered as acceptable in humans, is capable of modifying the gut microbiota in rat pups, in particular before puberty (PND 31).

4. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. In this publication there was no clinical evidence of alterations in activity or behavior in pups. Body weight, water and feed consumption both in dams and pups were no different across the groups. Litter sizes were fully comparable among groups. To identify changes in microbes with multiple analyses in groups of animals is not unexpected and not necessarily indicative of a specific effect of the active substance. Changes within all rats due to maturation are greater than the differences between treatment groups. Moreover there are several points limiting the significance of the results: 1) information to calculate dose is not in the paper and seems intentional, 2) ADI is not the same as exposure which averages 1% of the ADI, and clinical signs were by definition not observed at the NOAEL which is 100-fold greater than the ADI. Animals in these toxicity studies had gut microbes, 3) Claims of exposure via milk are unfounded. The statistical analysis results in some differences but they do not put these changes into the context of whether they are normal.

Only 1 dose tested, source of the test substance not reported.

The exact composition of the Roundup formulation tested is not stated in the paper (a.i. content, source, surfactant system / co-formulants). It is therefore not possible to confirm whether the product used is the representative glyphosate formulation MON 52276 relevant for the glyphosate EU renewal. Furthermore, in the absence of a concurrent control for each to the component of the formulation, it is not possible to conclude whether the observed effects claimed to be secondary to exposure to glyphosate are due to glyphosate exposure or of one of the other components. The uncertainty associated with whether the product contains polyethoxylated tallow amine (also polyoxyethyleneamine, POEA) or not, suggests that the findings in this paper should be treated with high level of caution.

Further points for clarification:

The effect of glyphosate and Roundup on the microbiota of Sprague Dawley rats and their offspring was investigated in this study. Glyphosate and Roundup were administered via drinking water to female rats during gestation day 6 to end of lactation. Offspring were similarly exposed to the aforementioned substances from the day of weaning until sacrifice either on PND 73 or on PND 125. The bacterial composition in F1 offspring was significantly different compared controls and between glyphosate and Roundup exposed F1 animals.

Assessment and conclusion by RMS:

In this study, possible effects of glyphosate and a glyphosate based formulation on the microbiome in rat during development was investigated with exposure via drinking water starting in dams on GD6 to end of lactation. After weaning the F1 generation was treated via drinking water until sacrifice and these animals were divided into two cohorts: 6-week and a 16-week cohort. Rat faecal samples were collected and analysed by 16S sequencing. No general toxicity was observed in the dams or offspring. No effect on the microbiome was observed in the dams. In the offspring, both glyphosate and the tested formulation altered the microbiota particularly before puberty (PND 31).

Gut microbiome is not part of the data requirements. In June 2020, EFSA published an editorial on exploring the need to include microbiomes into EFSA's scientific assessments (EFSA Journal 2020;18(6):e18061). In this editorial EFSA highlights that gut microbiome research is expected to play a relevant role in regulatory science and that further research is needed to enhance the understanding of the toxicological significance of microbiome-mediated metabolism of chemicals. Sequencing based tools and multi-omic approaches still need considerable work to refine and integrate them into study design as well as analytical standardisation.

The study is considered as supplementary information.
The study was carried out with glyphosate with unknown purity or batch number and a glyphosate based formulation for which no details are given. For the results in the formulation group, side effects caused by co-formulants cannot be excluded. Furthermore, it is unknown if the tested formulation contains polyethoxylated tallow amine (POEA). The route of exposure was via the drinking water, however, details on the actual doses the animals were exposed to are not given in the article. The study was not conducted according to any international guideline, only one dose was tested, individual data is missing, no historical control data or positive control included. Only maternal faeces was collected, samples from vagina or other body parts were not collected. Thus the role of maternal microbiota in the foetal development and its influence on pups intestinal colonization could not be examined.
Based on the aforementioned limitations the study is considered to be reliable with restrictions.

B.6.8.3. Studies on endocrine disruption

B.6.8.3.1. In vitro androgen receptor binding assay

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay
Report No	6500V-100334ARB
Document No	CTX-11-026
Guidelines followed in study	US OPPTS/OCSPP 890.1150 (2009)
Deviations from current test guideline (OCSPP 890.1150, 2009)	None
Previous evaluation	Yes, supplementary in EFSA peer review on endocrine disrupting properties (2017) due to missing information on the methods and results from the saturation binding experiments.

GLP/Officially recognised testing facilities	Yes
Comment on GLP	The control and reference substances were not characterized in accordance with GLP standards, nor was the stability under storage conditions at the test site determined in accordance with GLP standards. However, the substances were purchased from commercial suppliers, and the manufacturers' certificates of analysis were used to define the purity. Verification of the concentration, stability and homogeneity of the test, control, and reference substances in the test system were not determined in accordance with GLP. The test, control, and reference substances were soluble in their vehicles, dosing solutions were prepared immediately prior to use, and the control and reference substances met their performance criteria. Therefore, these exceptions do not affect the validity of the study.
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: The study is considered acceptable. Although information on the methods and results from the saturation binding experiments were not included in the study report, sufficient information was found in the EPA evaluation of the study to conclude on its reliability.

Executive summary
In this androgen receptor (AR) binding assay, ventral prostate cytosol from Sprague Dawley rats was used as source of AR to conduct both a saturation binding experiment and a competitive binding experiment. The saturation binding experiment was conducted to demonstrate that the AR isolated from rat prostate cytosol was present in reasonable amount and was functioning with appropriate affinity for the radiolabelled reference androgen (R1881) prior to routinely conducting AR competitive binding experiments. The competitive binding assay was conducted to measure the binding of a single concentration of [^{3}H]-R1881 (1 nM) in the presence of increasing concentrations of glyphosate (ranging from 10^{-10} to 10^{-3} M). The assay included dexamethasone as weak positive control and unlabelled R1881 as positive control (or non-labelled receptor ligand).

Three valid runs of the assay were completed. All concentrations were tested in triplicates. In the first valid, independent run, the mean specific binding of [^{3}H]-R1881 was >95% at every glyphosate concentration tested, except for 10^{-9} M, classifying it as a "non-binder" for this run. The mean specific binding of [^{3}H]-R1881 for 10^{-9} M glyphosate was 66.5%. However, this was due to a clear outlier where the hydroxyapatite pellet might have been lost. Excluding this value, a mean specific binding of 96.0% was determined, which concurs with the other runs. In the second and third valid runs, the mean specific binding of [^{3}H]-R1881 was >93% and >92%, respectively, classifying glyphosate as a "non-binder" in both runs. The weak positive control dexamethasone had a $LogIC_{50}$ of -4.6 M in each of the three runs. The $LogIC_{50}$ of R1881 was -9.0 M, -9.1 M and -9.0 M in the first, second, and third run, respectively.

Under the conditions of the present study, glyphosate was classified as a "non-binder" in all three valid runs resulting in a final classification of a non-binder to the androgen receptor.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White crystalline solid
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93% glyphosate acid 85.14% calculated glyphosate content

Stability of test compound:	Not reported. Expiry 2012-03-09

Vehicle and positive controls:

Solvent/vehicle used:	Low salt TEDG buffer (10 mM Tris, 1 mM sodium molybdate, 1.5 mM EDTA, 10% glycerol, and 1 mM DTT, pH 7.4)
Positive Control (non-labelled receptor ligand):	R1881 Source: Sigma-Aldrich Lot/Batch No.: 060M4638 Purity: 98% CAS No.: 965-93-5 Solvent used: DMSO
Weak Positive Control:	Dexamethasone Source: Sigma-Aldrich Lot/Batch No.: 1419230 Purity: 98.9% CAS No: 50-02-2 Solvent used: DMSO

Radioactive receptor ligand:

Identification:	[^{3}H]-R1881
Source:	Perkin-Elmer
Lot/Batch No.:	653698
Radiochemical Purity:	>97%
Specific activity:	85.1 Ci/mmol, certification date 2011-02-24
Solvent used:	Working Assay buffer = TEDG+PI buffer (10 mM Tris, 1 mM sodium molybdate, 1.5 mM EDTA, 10 % glycerol, 1 mM DTT, and 0.5 % Protease Inhibitor (PI) (v/v), pH 7.4)
Test system:	Rat ventral prostate cytosol was prepared from male Sprague Dawley rats (90 days of age) castrated at most one day prior delivery/ preparation. The prostate tissue was inspected for healthy appearance and excess fascia were trimmed if necessary. The prostates were weighed, placed in ice-cold TEDG buffer, and homogenized. The cytosol was isolated, pooled, and kept ice-cold. The protein concentration was 8.8 mg/mL.
Test concentrations:	Serial dilutions of glyphosate, the reference standard R1881, and the weak positive control dexamethasone were prepared to achieve the concentrations shown in the table below.

Table B.6.8.3.1-1: Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay ([redacted], 2012): Competitor final molar (M) concentrations in competitive binding assay[1, 2]

Positive control R1881	Weak positive control Dexamethasone	Test substance Glyphosate
1×10^{-6}	1×10^{-3}	1×10^{-3}
1×10^{-7}	1×10^{-4}	1×10^{-4}
1×10^{-8}	1×10^{-5}	1×10^{-5}
1×10^{-9}	1×10^{-6}	1×10^{-6}
1×10^{-10}	1×10^{-7}	1×10^{-7}
1×10^{-11}	1×10^{-8}	1×10^{-8}
-	1×10^{-9}	1×10^{-9}
-	1×10^{-10}	1×10^{-10}

[1] Each sample contained 10 µL of the solvent control, positive control (non-radiolabelled R1881), weak positive control, or test substance (with the exception of tubes used to evaluate non-specific binding, which contained 30 µL (100X) of non-radiolabelled R1881), 50 µL of triamcinolone acetonide, 30 µL [^{3}H]-R1881, and 300 µL of prostate cytosol.

[2] Each concentration of each chemical was run in triplicate.

B. STUDY DESIGN AND TEST PERFORMANCE

Experimental dates: 2011-10-25 to 2011-11-01

Saturation (radioligand) binding experiment
A saturation binding experiment measuring total and non-specific binding of [^{3}H]-R1881 was performed to demonstrate that the AR was present in reasonable amount and had the appropriate affinity for the R1881 ligand. The methods and results from the saturation binding experiment were not included in the final study report. However, a statement of the laboratory verifying that the saturation binding curve data were acceptable according to the US EPA OPPTS/OCSPP 890.1150 (2009) was included in the appendix of the study report.

Competitive binding experiment
The competitive binding experiment was performed to measure the binding of a single concentration of [^{3}H]-R1881 (final concentration 1 nM), to the AR in the presence of increasing concentrations of glyphosate. A summary of the experimental conditions for the competitive binding experiment is presented in the table below. The specific activity of [^{3}H]-R1881 was adjusted for decay over time prior to each run. The adjusted specific activity was 82.0, 82.0, and 81.9 Ci/mmol for the first, second, and third run of the assay, respectively. Each assay consisted of three independent runs conducted on three different days, and each run contained triplicates at each concentration. In addition to glyphosate, concentrations of R1881 (positive control) and dexamethasone (weak positive control) were also evaluated in the competitive binding experiment to ensure that the test system was behaving as expected.

Table B.6.8.3.1-2: Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay ([redacted], 2012): Summary of experimental conditions for competitive binding experiment

Source of receptor		Rat prostate cytosol
Concentration of radioligand		1 nM
Concentration of receptor		6.8 mg/mL, sufficient to bind 10 - 15 % of
Concentration of glyphosate (as serial dilutions)		100 pM to 1 mM
Temperature		4 ± 2 °C
Incubation time		16 - 20 h
Composition of assay buffer (TEDG+PI)	Tris	10 mM (pH 7.4)
	EDTA	1.5 mM
	Glycerol	10 % (v/v)
	Protease Inhibitor	0.5 % (v/v)
	DTT	1 mM
	Sodium molybdate	1 mM

C. DATA ANALYSIS

Classification of test material
The classification of glyphosate as a binder or non-binder was made on the basis of the average results of three non-concurrent runs, each of which met the performance criteria. Each run was individually classified as follows:
Binder: The lowest point on the fitted response curve within the range of the data was <50%.
Equivocal: The lowest point on the fitted response curve within the range of the data was >50% but <75%.
Non-Binder: The lowest point on the fitted response curve within the range of the data is >75% (i.e. <25% displacement of radioligand), or the data do not fit the model.

Descriptors for receptor binding

In circumstances when AR receptor binding was measured, the following parameters were determined.

- B_{max}: Maximal binding capacity
- K_d: Dissociation constant (nM), measures the affinity of the receptor for its natural ligand.
- IC_{50}: Concentration of the test substance at which 50 % of the radioligand is displaced from the receptor.

II. RESULTS AND DISCUSSION

A. SATURATION BINDING EXPERIMENT

Results from the saturation binding experiment were not provided in the final study report. However, the laboratory provided a statement in the appendix of the study report indicating that the saturation binding curve data were acceptable according to the US EPA OPPTS/OCSPP 890.1150 (2009). Reference control results included in the competitive binding experiment confirm that functional AR was present in sufficient amounts and functioning with appropriate affinity.

The following information was provided by the applicant in the M-document:
Summarised saturation binding data of the performing laboratory were submitted following a request by the US EPA (MRID 48843501). The dissociation constant (K_d) for [^{3}H]-R1881 was 0.613 ± 0.041 nM and the estimated B_{max} was 0.817 ± 0.049 fmol/100 µg protein for the single batch of prostate cytosol that was prepared. The mean and individual K_d values were below the range reported in the US EPA validation program (0.685 to 1.57 nM). However, confidence in these values is high according to the goodness of fit (R^2 = 0.957 – 0.984) and the small variation among runs.
This information could not be retrieved in the study report by the RMS, but is in line with the information provided in the ED evaluation of glyphosate made by US EPA (US EPA Report: Glyphosate EDSP Tier 1 Screening Results).

B. COMPETITIVE BINDING EXPERIMENT

Results from three valid runs of the competitive binding experiment are presented in the table and the figure below. In the first valid, independent run, the mean specific binding of [^{3}H]-R1881 was >95% at every soluble concentration tested for glyphosate, except for 10^{-9} M, classifying it as a non-binder for this run. The mean specific binding of [^{3}H]-R1881 for 10^{-9} M glyphosate was 66.5%; however, this was due to a clear outlier where it appeared the hydroxyapatite (HAP) pellet might have been lost. Excluding this value yielded a mean specific binding of 96.0%, which concurs with the other runs. In the second and third valid, independent runs, the mean specific binding of [^{3}H]-R1881 was >93 and >92%, respectively, classifying glyphosate as a non-binder in both of these runs. The weak positive control dexamethasone had a $LogIC_{50}$ of -4.6 M in each of the three runs. The $LogIC_{50}$ of R1881 was -9.0 M, -9.1 M and -9.0 M in the first, second, and third run, respectively, demonstrating low variability.

Table B.6.8.3.1-3: Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay ([redacted], 2012): Competitive binding assay of glyphosate with androgen receptor from rat prostate cytosol

Parameter	Substance	Run 1[1]	Run 2[1]	Run 3[1]	Mean ± SEM
r^2 (unweighted)	R1881	NR	NR	NR	NA
	Dexamethasone	NR	NR	NR	NA
	Glyphosate	NR	NR	NR	NA
Log IC_{50} (M)	R1881	-9.0	-9.1	-9.0	9.03 + 0.019
	Dexamethasone	-4.6	-4.6	-4.6	-4.6
	Glyphosate	NA	NA	NA	NA
IC_{50} (nM)	R1881	1.0	0.7947	1.0	0.9316 + 0.039
	Dexamethasone	25100	25100	25100	25100
	Glyphosate	NA	NA	NA	NA

[1] The mean is reported for the concurrent replicates within each run.
r^2 = Goodness of fit (r^2 is more appropriately expressed as a range, as opposed to a mean). For the test substance, these parameters could not be calculated.

NR = Not reported.
NA = Not applicable since binding was not evident.

Figure 6.8.3-1: Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay ([redacted], 2012): Percent [^{3}H]-R1881 bound to the androgen receptor in the presence of glyphosate (A), unlabelled R1881 (B, strong positive control), or dexamethasone (C, weak positive control) (mean of three valid runs ± SEM)

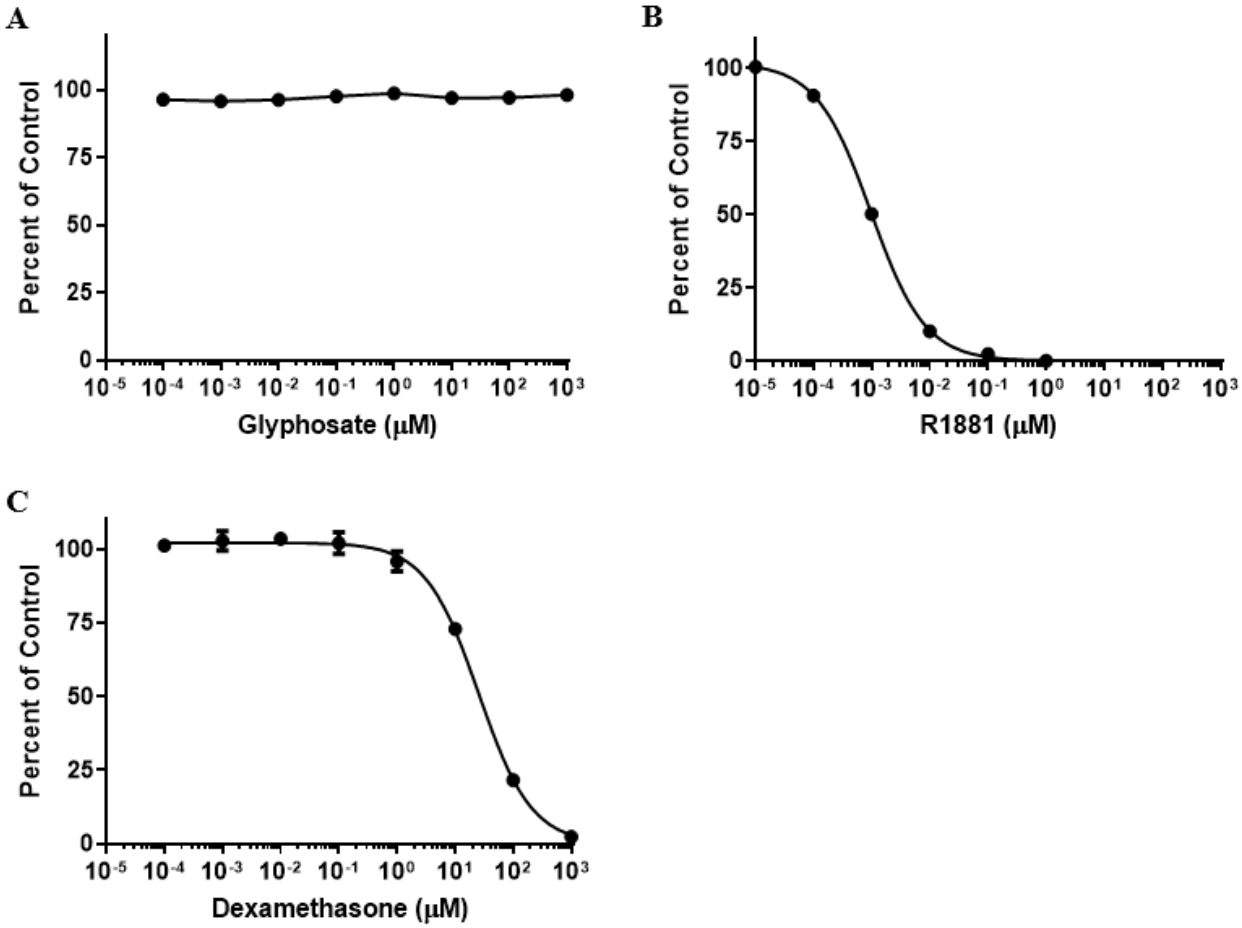


Table B.6.8.3.1-4: Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay ([redacted], 2012): Percent [^{3}H]-R1881 bound to the androgen receptor in the presence of glyphosate , unlabelled R1881, or dexamethasone (mean of three valid runs (standard deviation))

Test material	Concentration (Log[M])	Run 1		Run 2		Run 3	
Glyphosate	-3	95.5%	(5.8)	99.9%	(1.1)	99.0%	(2.7)
	-4	98.9%	(2.8)	98.9%	(0.7)	93.5%	(1.7)
	-5	98.6%	(0.9)	98.4%	(4.1)	94.1%	(1.6)
	-6	101.3%	(5.6)	97.2%	(3.5)	97.6%	(1.0)
	-7	99.4%	(4.8)	98.7%	(5.7)	94.9%	(1.1)
	-8	100.2%	(1.7)	96.4%	(1.9)	92.4%	(3.1)
	-9	66.5%	(51.1)	98.2%	(4.7)	93.5%	(6.6)
	-10	99.2%	(2.3)	93.8%	(8.1)	96.4%	(6.0)
R1881	-6	0.0%	(1.7)	0.0%	(1.0)	0.0%	(2.5)
	-7	2.1%	(2.5)	2.1%	(1.7)	2.8%	(1.9)

	-8	10.4%	(3.3)	11.4%	(2.5)	8.5%	(2.0)
	-9	48.4%	(6.5)	49.5%	(2.8)	52.7%	(4.3)
	-10	91.9	(2.8)	88.9%	(4.8)	90.9%	(2.6)
	-11	100.1%	(4.2)	102.1%	(1.2)	99.0%	(6.7)
Dexamethasone	-3	3.1%	(0.2)	4.8%	(1.8)	2.0%	(1.6)
	-4	21.3%	(0.)	22.9%	(0.6)	20.5%	(3.0)
	-5	71.6%	(6.8)	73.8%	(1.3)	73.2%	(0.9)
	-6	98.1%	(1.8)	97.3%	(2.7)	91.8%	(7.5)
	-7	106.1%	(1.7)	100.7%	(1.6)	99.1%	(3.3)
	-8	103.3%	(3.1)	104.7%	(2.4)	102.3%	(1.1)
	-9	105.4%	(1.8)	103.6%	(3.6)	99.0%	(1.0)
	-10	101.9%	(0.2)	100.3%	(3.0)	101.2%	(5.1)

III. CONCLUSIONS

Assessment and conclusion by applicant:
The study is performed in accordance with the current US OPPTS/OCSPP 890.1150 (2009). The study is therefore considered valid. In the conducted binding studies, glyphosate did not show any activity for androgen receptor binding in all three valid and independent runs of the assay. Thus, glyphosate was classified as a non-binder to the androgen receptor.

Assessment and conclusion by RMS:

The conclusion made by the applicant is agreed with.

B.6.8.3.2. In vitro estrogen receptor alpha transcriptional activation assay

1. Information on the study

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate
Report No	6500V-100334ERTA
Document No	CTX-11-028
Guidelines followed in study	US OPPTS/OCSPP 890.1300 (2009), OECD 455 (2009)
Deviations from current test guideline (OCSPP 890.1300, 2009) (OECD 455, 2016)	No major deviations to US OPPTS/OCSPP 890.1300 (2009). Hill slope value for 17α-estradiol, $LogPC_{50}$ and $LogPC_{10}$ value for 17α-methyltestosterone were outside the required range. Following deviations were noted to the current OECD 455 (2016): no ER antagonist assay was performed; demonstration of proficiency was stated but no data were included in study report; vehicle control (DMSO) for positive control was not tested; and some values (see above) were outside the required range. However, these deviations were not considered to affect the final outcome of the ER agonist assay.

Previous evaluation	Yes, considered to be supplementary in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The control and reference substances were not characterised in accordance with Good Laboratory Practice Standards, nor was the stability under storage conditions at the test site determined in accordance with Good Laboratory Practice Standards. However, the substances were purchased from commercial suppliers, and the manufacturers' certificates of analysis were used to define the purity. Verification of the test concentrations, stability and homogeneity of the test, control, and reference substances in the cell culture media were not determined in accordance with Good Laboratory Practice. The test, control, and reference substances were soluble in their vehicles, dosing solutions were prepared immediately prior to use and were shown to be solutions by visual inspection and the control and reference substances met their performance criteria. Therefore, these exceptions do not affect the validity of the study.
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: Study not acceptable due to deviation of the response of 17α-methyltestosterone from the acceptance criteria.

Executive summary
The ability of glyphosate to act as an agonist of human estrogen receptor alpha (hERα) was evaluated in an estrogen receptor (ER) transcriptional activation assay using the hERα-HeLa-9903 cell line. In two valid runs, stably-transfected hERα-HeLa-9903 cells were exposed to glyphosate at concentrations of 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M in cell culture medium. Preliminary assessments showed that glyphosate concentrations up to 10^{-3} M did not result in any precipitation or cytotoxicity (≥20 % reduction in cell viability). The cells were exposed to the test substance for 24 ± 2 hours and luciferase activity was measured. Each assay run included 17β-estradiol as a positive control and strong estrogen reference substance, 17α-estradiol as a weak estrogen reference substance, 17α-methyltestosterone as a very weak agonist reference substance, and corticosterone as a negative control. The positive control and reference substances induced the appropriate responses, with minor exceptions (Hill slope value for 17α-estradiol, $LogPC_{50}$ and $LogPC_{10}$ value for 17α-methyltestosterone were slightly outside the required range), and confirmed the sensitivity and performance of the assay.

Glyphosate demonstrated no evidence of interaction with the hERα over the concentration range tested in the HeLa-9903 model system, showing less than 10% of the induction potential of 17β-estradiol at the highest concentration tested.

Thus, glyphosate was not identified as an agonist of human estrogen receptor alpha (hERα) under the conditions of the present study.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White crystalline solid
Lot/Batch No.:	GLP-1103-21149-T

Purity:	95.93% glyphosate acid 85.14% calculated glyphosate content
Stability of test compound:	Not reported. Expiry: 2012-03-09
Vehicle, positive controls, and negative control:	
Solvent/vehicle used:	HeLa-9903 cell culture medium
Strong Positive Control (PC) estrogen:	17β-estradiol Source: Sigma-Aldrich Lot/Batch No.: 110M0138V Purity: 100% CAS No.: 50-28-2 Solvent used: DMSO
Weak PC estrogen:	17α-estradiol Source: Sigma-Aldrich Lot/Batch No.: 041M4065V Purity: 99.72% CAS No.: 57-91-0 Solvent used: DMSO
Negative Control (NC):	Corticosterone Source: Sigma-Aldrich Lot/Batch No.: BCBC6322V Purity: 99.2% CAS No.: 50-22-6 Solvent used: DMSO
Very Weak Agonist:	17α-methyltestosterone Source: Sigma-Aldrich Lot/Batch No.: 060M1543V Purity: 99% CAS No.: 58-18-4 Solvent used: DMSO
Test system:	Stably-transfected hERα-HeLa-9903 cells were obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank, Osaka 567-0085, Japan. Cells were free of mycoplasma infection and were maintained in Eagle's Minimum Essential Medium (EMEM) without phenol red, supplemented with 60 mg/L of kanamycin (antibiotic) and 10 % dextran coated-charcoal-stripped foetal bovine serum (DCC-FBS), in a CO_2 incubator (5 % CO_2) at 37 ± 1 °C. The cells used in the preliminary study, first valid run, and second valid run of the assay were passages 26, 27, and 16 (a new vial from cryopreservation), respectively.
Test concentrations:	Six concentrations each of glyphosate, 17β-estradiol, 17α-estradiol, corticosterone, and 17α-methyltestosterone were included in each valid run of the assay as shown in the table below.

Table B.6.8.3.2-1: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate ([redacted], 2012): Concentration range of glyphosate and reference substances in the ER transactivation assay

Reference substance	CAS No.	Concentration range	Class
Glyphosate	1071-83-6	10^{-10} - 10^{-3}	Test substance
17β-estradiol	50-28-2	10^{-14} - 10^{-8}	Strong estrogen
17α-estradiol	57-91-0	10^{-12} - 10^{-6}	Weak estrogen
Corticosterone	50-22-6	10^{-10} - 10^{-4}	Negative control
17α-methyltestosterone	58-18-4	10^{-11} - 10^{-5}	Very weak estrogen

B. STUDY DESIGN AND TEST PERFORMANCE

Experimental dates: 2011-09-20 to 2011-10-21

Preliminary cytotoxicity/ precipitation assays
In order to identify a suitable top concentration for use in the ER transcriptional activation assays, preliminary precipitation and cytotoxicity assays were conducted with glyphosate. Cytotoxicity (i.e. ≥20 % reduction in cell viability) was assessed using a two-read propidium iodide uptake assay at the following glyphosate concentrations: $10^{-6\,5}$, 10^{-6}, $10^{-5\,5}$, 10^{-5}, $10^{-4\,5}$, 10^{-4}, $10^{-3\,5}$, and 10^{-3} M; 125 µM digitonin was used as a positive control for cell death. No precipitation or cytotoxicity was observed by visual inspection at the highest glyphosate test concentration of 10^{-3} M.

ER transcription activation assay
The hERα-HeLa-9903 cells were plated in a 96-well culture plate at a density of ~1 x 10^4 cells/100 µL/well. After a 3-hour (minimum, typical incubation 3-4 hr) post-seeding incubation period, the medium was replaced with 150 µL/well of culture medium containing vehicle control (VC), glyphosate, or the reference chemicals at the concentrations identified in the table above. The final concentration of DMSO in the culture medium for the reference substances was held constant at 0.1% (v/v). Glyphosate dosing solutions were prepared in tissue culture medium. The tissue culture plates were incubated in an incubator under an atmosphere of 5% CO_2 and at 37 ± 1 °C for 24 ± 2 hours prior to measuring luciferase activity.

C. DATA ANALYSIS

Relative transcriptional activity
Relative transcriptional activity of glyphosate was expressed as percent of the maximally inducing concentration of the positive control, 1 nM 17β-estradiol.

Data interpretation
The data were interpreted according to the following steps (according to the test guideline):

1. Where appropriate, $LogPC_{50}$, $LogPC_{10}$, $LogEC_{50}$ and Hill slope values were calculated using XLfit from IDBS version 5.2.0.0 (Surrey, UK).
2. For the test substance, the maximum response relative to the positive control (RPC_{max}) was determined. In each individual run of the transcriptional activation assay, if RPC_{max} was <10 %, the test substance was considered to give a negative response for hERα agonism.
   RPC_{max} = Maximum level of response induced by a test chemical, expressed as a percentage of the response induced by the positive control (17β-estradiol at 1 nM) on the same plate.
3. For each individual run of the transcriptional activation assay, the acceptability of the data was evaluated using the following criteria:
   a. The mean normalized luciferase signal of the PC (1 nM 17β-estradiol) should be at least 4-fold that of the mean VC on each plate.
   b. The fold induction corresponding to the PC_{10} value of the concurrent PC is greater than 1 + 2 SD of the fold induction value (=1) of the VC. [PC_{10} = Concentration of a test chemical at which the response is 10 % of the response induced by the positive control (17β-estradiol at 1 nM) in each plate.
   c. The results of the four reference substances should be within acceptable ranges.
4. If the acceptability criteria outlined above were met, that run of the transcriptional activation assay was considered to be valid.
5. The test substance was considered negative if RPC_{max} <10 % in at least two valid runs of the transcriptional activation assay. The test substance was considered positive if RPC_{max} ≥10 % in at least two valid runs of the transcriptional activation assay.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY/ PRECIPITATION ASSAYS
Cytotoxicity for glyphosate did not exceed the defined threshold for cytotoxicity of 20% reduced cell viability

and precipitation of glyphosate was not observed at the highest test concentration of 10^{-3} M. Therefore, the final concentrations of glyphosate tested in the two valid runs of the transcriptional activation assay were 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} M.

B. ER TRANSCRIPTION ACTIVATION ASSAY

In the main assays, cytotoxicity (≥20 % reduction in cell viability) and precipitation were not observed at any of the tested concentrations of glyphosate. In the two valid, independent runs of the assay, the highest mean induction values for glyphosate (RPC_{max}) were 2.4 ± 1.4% and 0.8 ± 0.3% over the concentration range tested in the first and second run (see table and figure below), respectively. These mean induction values were clearly below the criterion for a positive response (i.e. maximum response relative to the positive control, RPC_{max} of glyphosate was <10% of the induction produced by 1 nM 17β-estradiol) over the concentration range tested. The mean response for 1 nM 17β-estradiol showed a concentration-dependent response with mean induction of approximately 120-fold.

In both valid runs of the assay, the positive control and reference substances induced the appropriate responses meeting the performance standards with some exceptions as follows:

- In the second valid run of the assay, the Hill Slope value for 17α-estradiol was slightly higher than the guideline suggested criteria (2.3 compared to the range of 0.9 to 2.0).
- In both valid runs of the assay, the Log PC_{50} value for 17α-methyltestosterone was not reached. However, induction was able to reach 40.8 - 42.6% of the positive control.
- In both valid runs of the assay, the Log PC_{10} value for 17α-methyltestosterone was slightly higher than the guideline suggested criteria (-5.9 for both valid runs, compared to the range of -6.2 to -8.0).

These exceptions were considered minor by the study author and not to have affected interpretation and final outcome of the assay results, as the responses of 17β-estradiol, 17α-estradiol, 17α-methyltestosterone, and corticosterone were characteristic of a strong estrogen, a weak estrogen, a weak agonist, and a negative compound, respectively. The US EPA convened a Scientific Advisory Panel (SAP) in 2013 to comment on the significance of minor deviations for performance criteria for the reference substances. The SAP concluded that minor deviations from the performance criteria do not affect the validity of these studies (***Note RMS: the applicant is asked to provide support for this statement, the RMS considers that the deviation from the acceptance criteria for 17α-methyltestosterone may indicate a decreased sensitivity of the study for weak agonists).***

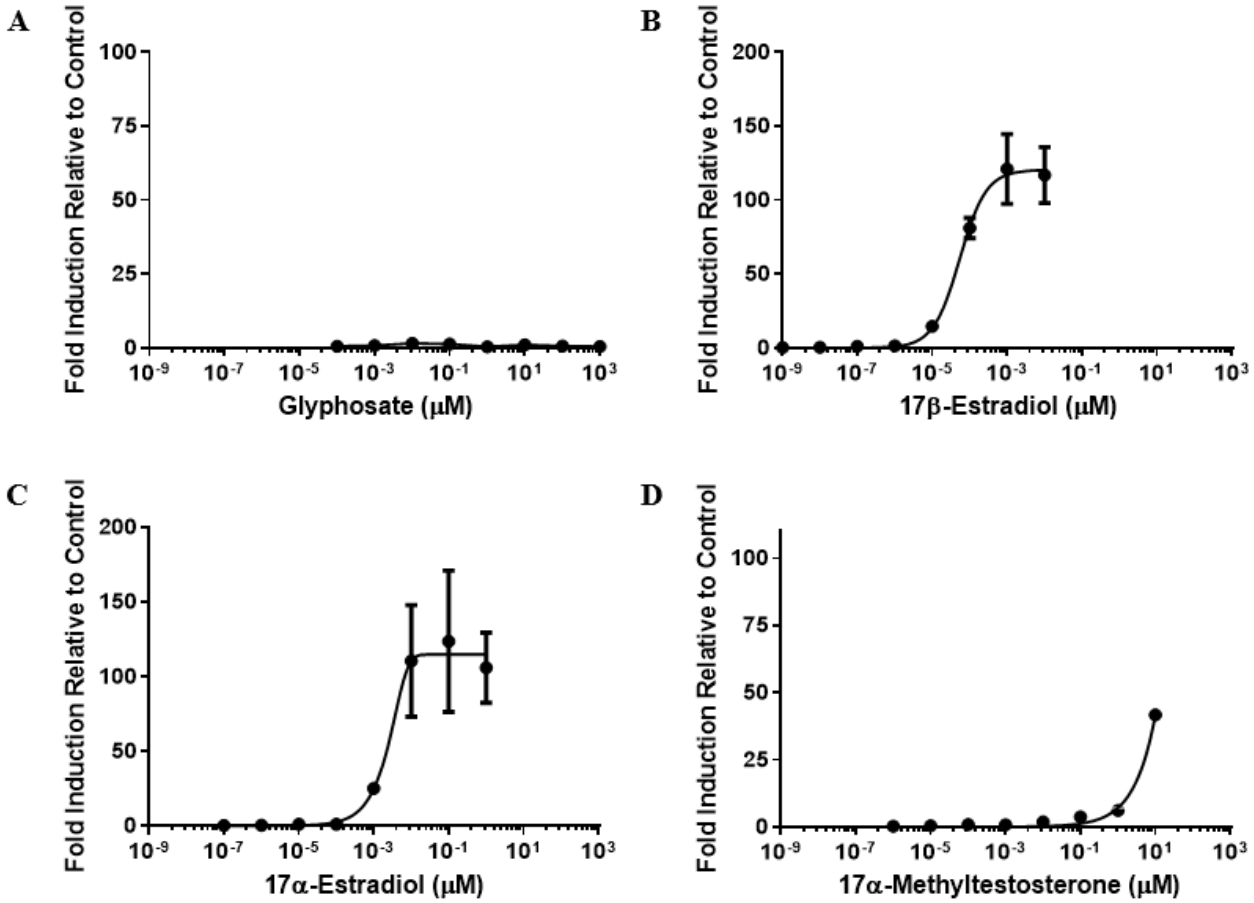
Table B.6.8.3.2-2: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate ([redacted], 2012): Relative transcriptional activation (RTA) induced by glyphosate

Parameter	RTA (mean ± SD); % of Positive Control (PC)			
	Run 1		Run 2	
Glyphosate concentration (M)	Mean	± SD	Mean	± SD
10^{-3}	0.5	0.8	0.5	0.3
10^{-4}	1.3	1.1	0.3	0.2
10^{-5}	2.4	1.4	0.7	0.3
10^{-6}	1.8	1.1	0.8	0.3
10^{-7}	0.8	1.0	0.0	0.3
10^{-8}	1.8	1.5	0.3	0.3
10^{-9}	1.2	1.6	0.0	0.3
10^{-10}	1.1	1.0	-0.1	0.3
RPC_{max}	2.4 ± 1.4 %		0.8 ± 0.3 %	

PC_{10}	NA	NA

NA = not applicable and could not be calculated because all responses were below a PC_{10} indicating that glyphosate did not induce estrogen receptor-mediated transactivation.

Figure B.6.8.3.2-1: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate ([redacted] 2012): Percent induction following 24 ± 2 h of exposure to glyphosate (A), 17β-estradiol (B, strong positive control), 17 α-estradiol (C, moderately strong positive control), or 17 α-methyltestosterone (D, weak positive control) (mean of two valid assays ± SEM)



III. CONCLUSIONS

Assessment and conclusion by applicant:

The study is performed in accordance with the current US OPPTS/OCSPP 890.1150 (2009) with no major deviations. The study is also performed in accordance with the current OECD 455 (2016), however the following deviations were noted: no ER antagonist assay was performed; demonstration of proficiency was stated but no data were included in study report and vehicle control (DMSO) for positive control was not tested. These deviations were not considered to affect the final outcome of the ER agonist assay. The study is therefore considered valid. Based on the results of the two valid transcriptional activation assays performed in the HeLa-9903 model system, glyphosate did not act as agonist of the human estrogen receptor alpha (hERα) over the concentration range tested (maximum concentration 10^{-3} M). The highest mean induction value of glyphosate was 2.4 ± 1.4 % (RPC_{max}), which is well below 10 % of the response of the positive control (1 nM 17β-estradiol) and represents a negative response for hERα agonism.

Thus, glyphosate was not identified as an agonist of human estrogen receptor alpha (hERα) under the conditions of the present study.

Assessment and conclusion by RMS:

Glyphosate does not show an agonistic effect on the human estrogen receptor alpha (hERα). However, the study is considered to be unacceptable and supplementary only due to the deviation of the response of 17α-methyltestosterone from the acceptance criteria.

This conclusion is in line with the previous EU evaluation (addendum 2 to the RAR, 2017).

B.6.8.3.3. In vitro estrogen receptor binding assay

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay
Report No	6500V-100334ERB
Document No	CTX-11-029
Guidelines followed in study	US OPPTS/OCSPP 890.1250 (2009)
Deviations from current test guideline (OCSPP 890.1250, 2009) (OECD 493, 2015)	None to US EPA OPPTS/OCSPP Test Guideline 890.1250 (2009). Current OECD 493 (2015) is not applicable (present study obtained the estrogen receptor from rat uterine cytosol preparations; OECD 493 (2015) applies to two fully validated human recombinant receptor ERα (hrERα) *in vitro* test methods).
Previous evaluation	Yes, supplementary in EFSA peer review on endocrine disrupting properties (2017) due to missing information on the methods and results from the saturation binding experiments.
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The control and reference substances were not characterised in accordance with GLP standards, nor was the stability under storage conditions at the test site determined in accordance with GLP standards. However, the substances were purchased from commercial suppliers, and the manufacturers' certificates of analysis were used to define the purity. Verification of the test concentrations, stability and homogeneity of the test, control, and reference substances in the test system were not determined in accordance with GLP. The test, control, and reference substances were soluble in their vehicles, dosing solutions were prepared immediately prior to use, and the control and reference substances met their performance criteria. Therefore, these exceptions do not affect the validity of the study.
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: Study acceptable. Although information on the methods and results from the saturation binding experiments were not included in the study report sufficient information was found in the EPA evaluation of the study to conclude on its reliability.

Executive summary

In this estrogen receptor binding assay, the ability of glyphosate to interact with the estrogen receptor (ER) was evaluated. Cytosol isolated from Sprague Dawley rat uteri was used as source of ER to conduct a saturation binding experiment and a competitive binding experiment according to US EPA OPTTS test guideline 890.1250 (2009). The saturation binding experiment was conducted to demonstrate that the ER contained in the rat uterine

cytosol was present in reasonable amount and was functioning with appropriate affinity for the radiolabelled reference estrogen, [^{3}H]-17β-estradiol, prior to routinely conducting the ER competitive binding experiment. The competitive binding experiment was conducted to measure the binding of a single concentration of [^{3}H]-17β-estradiol (1 nM) to the ER, in the presence of increasing concentrations of glyphosate (ranging from 10^{-10} - 10^{-3} M). All concentrations were tested in replicates of three. The duration of incubation was 16 - 20 hours at 4 °C.

Four runs were conducted, however, the first run was considered invalid because the results for 17β-estradiol (positive control) did neither fit the guideline-established acceptance criteria nor did they fall within the range of historical data. Mean specific binding was >81%, >95%, and >111% at every concentration of glyphosate tested in the first, second, and third valid runs of the assay, respectively, and averaged >100% across the concentration range. Glyphosate showed no evidence of competing with estradiol for the ER and was classified as "non-interacting" for all three valid runs.

The weak positive control, 19-norethindrone met the validity criteria for each of the runs, and each assay had a Log IC_{50} value of -5.5 M. The Log IC_{50} values for 17β-estradiol also met the validity criteria and were -9.1 M, -9.0 M and -8.9 M in the first, second, and third valid runs, respectively. The negative control octyltriethoxysilane was correctly concluded not to interact with the ER.

Based on the results of the present Estrogen Receptor Binding Assay, glyphosate is not a ligand for the ER and is classified as not-interacting with the ER.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White crystalline solid
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93% glyphosate acid 85.14% calculated glyphosate content
Stability of test compound:	Not reported. Expiry: 2012-03-09
Vehicle, positive controls, and negative control:	
Solvent/vehicle:	Assay buffer, i.e. TEDG+PI buffer (composition: 10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 0.5 % Protease Inhibitor (PMSF) (v/v), 10 % glycerol, pH ~7.4)
Positive Control (non-labelled receptor ligand):	17β-estradiol Source: Sigma-Aldrich Lot/Batch No.: 110M0138V Purity: 100% CAS No.: 50-28-2 Solvent used: DMSO
Weak Positive Control:	19-norethindrone Source: Sigma-Aldrich Lot/Batch No.: 030M1359V Purity: 99% CAS No.: 68-22-4 Solvent used: DMSO
Negative Control:	Octyltriethoxysilane Source: Sigma-Aldrich Lot/Batch No.: 24996KK Purity: 99.34% CAS No.: 2943-75-1 Solvent used: DMSO

Radioactive receptor ligand:

Identification:	[^{3}H]-17β-estradiol
Source:	Perkin-Elmer
Lot/Batch No.:	650702
Radiochemical purity:	>97%
Specific activity:	130.2 Ci/mmol, certification date: 2011-05-06
Solvent used:	DMSO
Test system:	Rat uterine cytosol prepared from 12 - 13-week old female Sprague Dawley rats ovariectomized seven days prior to being euthanized. Uterine tissue was inspected for signs of residual ovarian tissue after ovariectomy. The uteri were weighed, placed in ice-cold TEDG+PI buffer, and homogenized. The cytosol was isolated, pooled and kept ice-cold. The protein concentration of the cytosol was 1.1 mg/mL.
Test concentrations:	Dilutions of glyphosate, 19-norethindrone, octyltriethoxylsilane, 17β-estradiol are shown in the table below.

Table B.6.8.3.3-1: Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay ([redacted], 2012): Competitor final molar (M) concentrations in competitive binding assay [1, 2]

Positive Control 17β-Estradiol	Weak Positive Control 19-Norethindrone	Negative Control Octyltriethoxysilane	Test Substance Glyphosate
1.0×10^{-7}	1.0×10^{-4}	1.0×10^{-3}	1.0×10^{-3}
1.0×10^{-8}	3.16×10^{-5}	1.0×10^{-4}	1.0×10^{-4}
3.16×10^{-9}	3.16×10^{-6}	1.0×10^{-5}	1.0×10^{-5}
1.0×10^{-9}	1.0×10^{-6}	1.0×10^{-6}	1.0×10^{-6}
3.16×10^{-10}	3.16×10^{-7}	1.0×10^{-7}	1.0×10^{-7}
1.0×10^{-10}	1.0×10^{-7}	1.0×10^{-8}	1.0×10^{-8}
1.0×10^{-11}	3.16×10^{-8}	1.0×10^{-9}	1.0×10^{-9}
-	3.16×10^{-9}	1.0×10^{-10}	1.0×10^{-10}

[1] Each sample contains: 10 µL of either the test substance, weak positive control, negative control, solvent control, or positive control; 390 µL of TEDG+PI buffer with [^{3}H]-17β-estradiol; and 100 µL of uterine cytosol (with ER), for a total of 500 µL.
[2] Each concentration of each chemical was run in triplicate.

B. STUDY DESIGN AND TEST PERFORMANCE

Experimental dates: 2011-11-03 to 2011-12-03

Saturation (radioligand) binding experiment
A saturation binding experiment to measure total and non-specific binding of [^{3}H]-17β-estradiol was performed to demonstrate that the ER was present in reasonable concentrations and had appropriate affinity for the native ligand. The methods and results from the saturation binding experiment were not included in the final study report. However, a statement of the laboratory verifying that the saturation binding curve data were acceptable according to the US OPPTS/OCSPP 890.1250 (2009) was included in the appendix of the study report.

Competitive binding experiment
The competitive binding experiment was performed to measure the binding of a single concentration of [^{3}H]-17β-estradiol (1 nM) to the ER in the presence of increasing concentrations of glyphosate. A summary of the experiment conditions for the competitive binding experiment is included in the table below. The specific activity of [^{3}H]-17β-estradiol was adjusted for decay over time prior to each run. The adjusted specific activity was 126.6, 126.5, and 126.1 Ci/mmol for the first, second, and third valid runs of the assay, respectively.

Each assay consisted of three independent valid runs, which were conducted on three different days, and each run of the assay consisted of triplicates at each test concentration. In addition to glyphosate, concentrations of 17β-estradiol, 19-norethindrone, and octyltriethoxysilane were also evaluated in the competitive binding experiment to ensure that the test system was behaving as expected.

Table B.6.8.3.3-2: Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay ([redacted], 2012): Summary of conditions for competitive binding experiment

Source of receptor		Rat uterine cytosol
Concentration of radioligand		1 nM
Concentration of receptor		Sufficient to bind 10 - 15% of radioligand
Concentration of glyphosate (as serial dilutions)		100 pM to 1 mM
Temperature		4 ± 2°C
Incubation time		16 - 20 hours
Composition of assay buffer (TEDG+PI buffer)	Tris	10 mM (pH 7.4)
	EDTA	1.5 mM
	Glycerol	10% (v/v)
	Protease Inhibitor (Phenylmethylsulfonyl fluoride (PMSF))	0.5% (v/v)
	DTT	1 mM

C. DATA ANALYSIS

Classification of test material

The test material was classified based on the average of three valid runs of the assay. Each run was individually classified as follows:

Interactive = Lowest point on the fitted curve within the range of the data is <50 % (i.e. >50 % of the radiolabelled estradiol has been displaced from the ER).

Not interactive = There are usable data points at or above 10^{-6} M and either the lowest point on the fitted response curve within the range of the data is >75 % (i.e. <25 % of the radiolabelled estradiol has been displaced from the ER), or a binding curve cannot be fitted and the lowest average percent binding among concentration groups in the data is >75 %.

Equivocal up to the limit of concentrations tested = If there are no data points at or above a test chemical concentration of 10^{-6} M and either a binding curve can be fit but ≤50 % of the radiolabelled estradiol has been displaced from the ER, or a binding curve cannot be fit and the lowest average percent binding among concentration groups in the data is >50 %.

Equivocal = A run is classified as equivocal if it does not fall into any of the categories above.

Descriptors for receptor binding

In circumstances when ER receptor binding was measured, the following parameters were determined:

- B_{max}: Maximum specific binding number (fmol ER/100 µg cytosol protein) measures the concentration of active receptor sites.
- K_d: Dissociation constant (nM), measures the affinity of the receptor for its natural ligand.
- IC_{50}: Concentration of the test substance at which 50 % of the radioligand is displaced from the receptor.

II. RESULTS AND DISCUSSION

A. SATURATION BINDING EXPERIMENT

Results from the saturation binding experiment were not provided in the study report, although the laboratory provided a statement in the Appendix of the study report indicating that the saturation binding curve data were acceptable according to the respective US OPPTS/OCSPP 890.1250 (2009). Reference control results included in the competitive binding experiment confirmed that functional ER was present in sufficient amounts and

functioning with appropriate affinity.

The following information was provided by the applicant in the M-document:
Summarised saturation binding data from the performing laboratory were submitted following a request by the Agency (MRID 48843501). The protein concentrations used in the saturation binding runs varied between each run, and were approximately 3- to 6-fold greater than recommended in the test guideline (160 to 320 µg versus 50 ± 10 µg, respectively). The K_d for [^{3}H]-17β-estradiol was 0.331 ± 0.061 nM, and the estimated B_{max} was 74.55 ± 3.03 fmol/100 µg protein for the prepared rat uterine cytosol. The K_d for each run was within the expected test guideline range of 0.03 - 1.5 nM.
This information could not be retrieved in the study report by the RMS, but is in line with the information provided in the ED evaluation of glyphosate made by US EPA (US EPA Report: Glyphosate EDSP Tier 1 Screening Results).

B. COMPETITIVE BINDING EXPERIMENT
Four runs of the assay were conducted; however, the first run was considered invalid because the positive control 17β-estradiol did not meet the guideline-established acceptance criteria or was outside the range of historical control data. The remaining three runs met all acceptance criteria and are considered valid.

Results from the valid runs of the competitive binding experiment are presented in the table below. Specific binding was >81%, >95%, and >111% at every concentration of glyphosate tested in the first, second, and third valid runs of the assay, respectively, and averaged >100% across the concentration range (see figure below). Mean specific binding of the positive control, 17β-estradiol, and the weak positive control, 19-norethinodrone, met the guideline-established performance criteria for the assay in the three valid runs of the assay. The curve for the reference material showed that increasing concentrations of unlabelled 17β-estradiol displaced [^{3}H]-17β-estradiol in a manner consistent with one-site binding (see figure below). The Log IC_{50} of 17β-estradiol was -9.1 M, -9.0 M and -8.9 M in the first, second, and third valid run, respectively. 19-Norethindrone, had a Log IC_{50} of -5.5 M for each of the three valid runs. Confidence in these numbers is high due to the small variation.
Mean specific binding of octyltriethoxysilane, the negative control, was >88% at all concentrations except 10^{-3} M in the first and second valid assays, where the mean specific binding was 46.0% and 47.4%, respectively at 10^{-3} M. This phenomenon of lower mean specific binding observed in the first and second valid assays has previously been observed at the highest test concentration of octyltriethoxysilane and is associated with compound precipitation, rather than true competitive inhibition. Although precipitation was not specifically observed octyltriethoxysilane was added to the cytosol preparation containing ERs, an opaque protein slurry, which can make the identification of precipitation difficult to assess.

Table B.6.8.3.3-3: Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay ([redacted], 2012): Results of competitive binding assay of glyphosate with estrogen receptor from rat uterine cytosol

Parameter	Compound	Run 1[1]	Run 2[1]	Run 3[1]	Mean ± SEM
r^2 (unweighted)	17β-estradiol	NR	NR	NR	NA
	19-Norethinodrone	NR	NR	NR	NA
	Glyphosate	NR	NR	NR	NA
Log IC_{50} (M)	17β-estradiol	-9.1	-9.0	-8.9	-9.0 ± 0.03
	19-Norethinodrone	-5.5	-5.5	-5.5	-5.5
	Glyphosate	NA	NA	NA	NA
IC_{50} (nM)	17β-estradiol	0.7947	1	1.26	1.02 ± 0.074
	19-Norethinodrone	3160	3160	3160	3160
	Glyphosate	NA	NA	NA	NA

[1] The mean is reported for the concurrent replicates within each run.
r^2 = Goodness of fit (r^2 is more appropriately expressed as a range, as opposed to a mean). For the test substance, these parameters could not be calculated.
NR = Not reported.
NA = Not applicable since binding was not evident.

Figure B.6.8.3.3-1: Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay ([redacted], 2012): Relative amount of [^{3}H]-17β-estradiol bound to the estrogen receptor in the presence of glyphosate (A), unlabelled 17β-estradiol (B, positive control), 19-norethindrone (C, weak positive control), or octyltriethoxysilane (D, negative control) (mean of three valid assays, ± SEM)

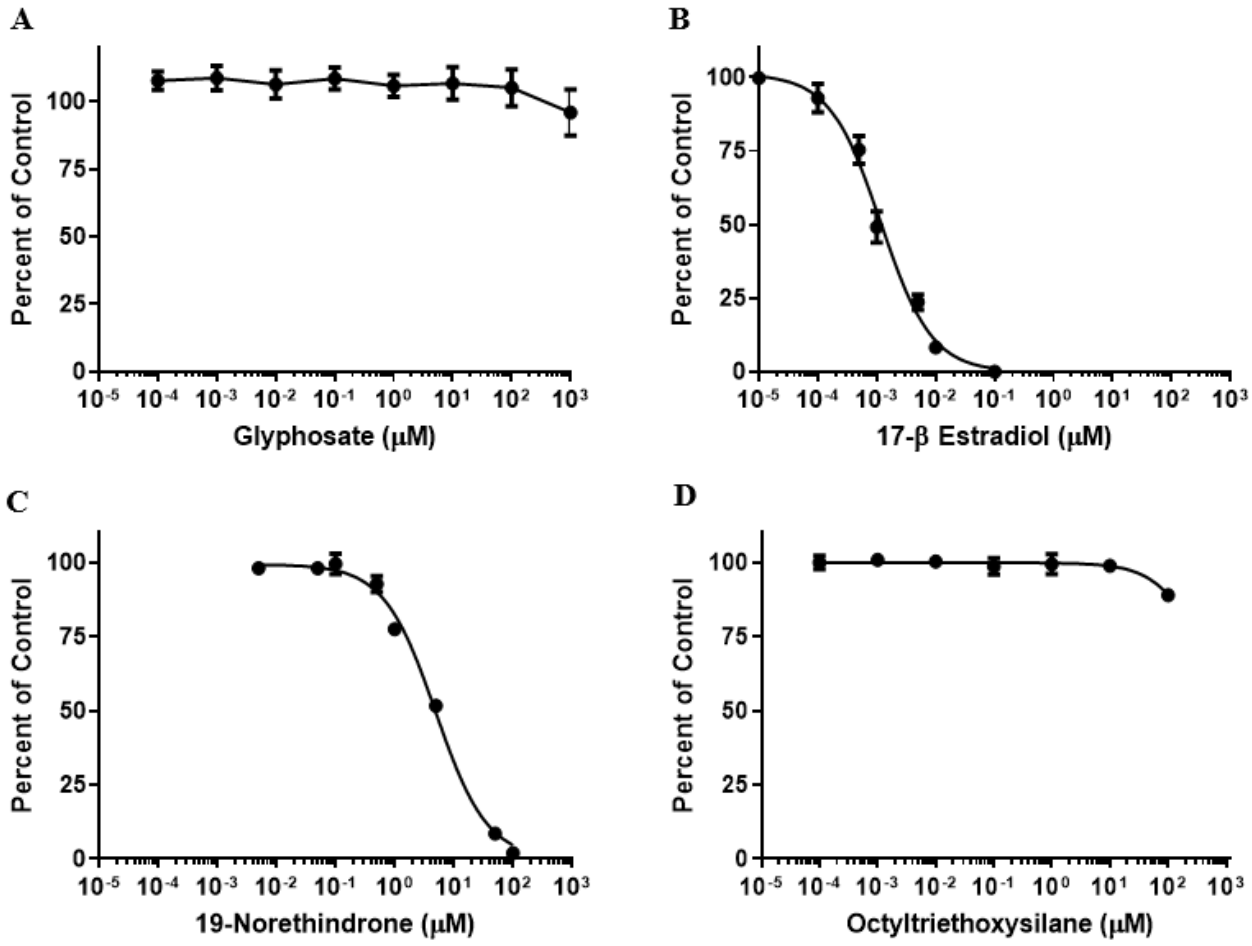


Table B.6.8.3.1-4: Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay ([redacted], 2012): Relative amount of [^{3}H]-17β-estradiol bound to the estrogen receptor in the presence of glyphosate, unlabelled 17β-estradiol (positive control), 19-norethindrone (weak positive control), or octyltriethoxysilane (negative control) (mean of three valid runs (standard deviation))

Test material	Concentration (Log[M])	Run 1		Run 2		Run 3	
Glyphosate	-3	81.6	(12.7)	95.4	(4.5)	111.1	(1.2)
	-4	94.5	(4.9)	103.4	(1.8)	117.8	(3.1)
	-5	95.2	(5.9)	109.5	(2.2)	115.9	(2.3)
	-6	98.7	(1.1)	106.1	(2.6)	113.0	(0.7)
	-7	102.6	(3.6)	106.7	(0.9)	116.6	(1.4)
	-8	96.7	(0.9)	108.1	(2.4)	114.5	(3.1)
	-9	101.2	(1.8)	108.3	(0.9)	116.9	(3.9)
	-10	101.5	(1.4)	108.7	(1.9)	113.4	(1.8)
Estradiol (NSB)	-7	0.0	(0.1)	0.0	(0.3)	0.0	(0.5)

	-8	7.0	(0.1)	8.2	(0.2)	9.7	(1.6)
	-8.5	21.6	(0.5)	22.9	(1.5)	26.6	(2.0)
	-9	44.1	(1.4)	48.7	(2.0)	54.7	(2.9)
	-9.5	70.1	(0.6)	77.2	(2.5)	78.9	(3.2)
	-10	87.5	(2.6)	94.8	(4.8)	96.8	(0.4)
	-11	99.0	(0.8)	101.8	(1.7)	98.6	(3.1)
19-norethindrone	-4	2.2	(0.1)	1.6	(0.4)	2.1	(0.2)
	-4.5	8.8	(0.5)	7.5	(0.3)	9.4	(0.7)
	-5.5	50.6	(4.5)	50.9	(0.4)	53.5	(2.0)
	-6	75.3	(2.2)	77.8	(2.2)	79.5	(1.6)
	-6.5	89.8	(2.8)	93.4	(2.3)	95.0	(3.2)
	-7	95.6	(4.3)	102.1	(0.3)	100.7	(2.0)
	-7.5	97.0	(3.4)	98.0	(4.3)	99.0	(0.6)
	-8.5	97.9	(1.0)	97.9	(3.8)	98.3	(3.3)
Octyltriethoxysilane	-3	46.0	(2.6)	47.4	(6.7)	98.1	(1.5)
	-4	89.1	(1.6)	88.8	(1.9)	100.5	(2.8)
	-5	98.2	(1.2)	99.5	(2.5)	101.8	(1.2)
	-6	97.0	(1.9)	101.8	(1.0)	101.5	(1.2)
	-7	96.7	(1.6)	100.6	(1.0)	100.8	(7.6)
	-8	99.2	(1.9)	101.5	(1.9)	104.9	(4.5)
	-9	100.5	(1.9)	101.3	(3.3)	102.8	(3.5)
	-10	98.3	(3.1)	101.6	(1.3)	104.4	(1.7)

III. CONCLUSIONS

Assessment and conclusion by applicant:
The study is performed in accordance with the current US OPPTS/OCSPP 890.1250 (2009). Deviations from the current OECD 493 (2015) is not applicable as the present study obtained the estrogen receptor from rat uterine cytosol preparations and the OECD 493 (2015) applies to two fully validated human recombinant receptor ERα (hrERα) *in vitro* test methods. The study is therefore considered valid. No interaction of glyphosate with the estrogen receptor was determined in the performed saturation and competitive binding experiment. Based on these results, glyphosate was classified as "non-interacting" in all three valid independent runs of the Estrogen Receptor Binding Assay. Thus, under the conditions reported, glyphosate was assigned a final classification of "non-interacting" for binding to the estrogen receptor.

Assessment and conclusion by RMS:

The conclusion made by the applicant is agreed with.

B.6.8.3.4. In vitro aromatase assay

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Human Recombinant Aromatase Assay
Report No	6500V-100334AROM

Document No	CTX-11-027
Guidelines followed in study	US OPPTS/OCSPP 890.1200 (2009)
Deviations from current test guideline (OCSPP 890.1200, 2009)	None
Previous evaluation	Yes, supplementary in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The positive control substance was not characterised in accordance with GLP standards, nor was the stability under storage conditions at the test site determined in accordance with GLP standards. However, the substance was purchased from commercial supplier, and the manufacturers' certificate of analysis was used to define the purity. Concentrations of test substance and control substances were not verified using analytical methods. In view of the short-term nature of studies of this type, no analyses of stability, homogeneity or achieved concentration(s) were conducted on preparations of the test substance or positive control chemicals, either before or after the treatment phase. This exception is not considered to have affected the integrity of the study and is not required in the US OPPTS 890.1200 guideline. For the reference control compounds, stability was demonstrated by an appropriate response in the assay system. Proficiency testing done to certify analysts was not performed under GLP and the results of proficiency testing are not included in the final report. However, the raw data was provided to US EPA by the test facility and support the expected designations of inhibitor or non-inhibitor for each of the proficiency chemicals (econazole, fenarimol, nitrofen, and atrazine) as well as the positive control (4-OH ASDN).
Acceptability/Reliability:	Conclusion GRG: Valid, category 2A Conclusion AGG: acceptable.

Executive summary

Glyphosate was evaluated for the potential to inhibit aromatase catalytic activity. In an *in vitro* aromatase (CYP19) assay, glyphosate was incubated with human recombinant aromatase (human CYP19 [Aromatase] and P450 reductase Supersomes^{TM}) and tritiated androstenedione ([^{3}H]ASDN) at concentrations of 10^{-10} M to 10^{-3} M for 15 minutes to assess the effect of glyphosate on aromatase activity. The solvent vehicle was 0.1 M sodium phosphate buffer for glyphosate, ethanol for ASDN, and dimethylsulfoxide (DMSO) for the positive control inhibitor 4-hydroxyandrostenedione (4-OH ASDN).

Aromatase activity was determined by measuring the amount of tritiated water produced at the end of a 15-minute incubation for each concentration of the used substances. Four independent runs of the assay were conducted; however, the first run was unacceptable because of incorrect standard preparation. Each run included a full activity control, a background activity control, a positive control series (10^{-10} M to 10^{-5} M) using the known inhibitor 4-OH ASDN in duplicate per concentration, and the test substance series (10^{-10} M to 10^{-3} M) in triplicate per concentration.

Aromatase activity in the full activity controls was 0.593 nmol/mg protein/min, which was greater than the minimum recommended aromatase activity of 0.1 nmol/mg protein/min. The response of the full activity controls and background controls was acceptable for each run.

For the positive control substance (4-OH ASDN), aromatase activity results were within the recommended ranges for the performance criteria. The estimated Log IC_{50} for 4-OH ASDN averaged -7.29 M and the Hill slope was -0.96.

For glyphosate, mean aromatase activity was 99.67 ± 1.86 % of vehicle control at the lowest tested concentration

of 10^{-10} M and 109.3 ± 5.53 % of the vehicle control at the highest tested concentration of 10^{-3} M. The average aromatase activity was ≥99.67 % of the control at all tested glyphosate concentrations for all runs. Hill slope estimates were not determined for glyphosate because it never achieved >25 % inhibition. Thus, in three independent runs of the assay, increasing concentrations of glyphosate showed no decrease in aromatase activity.

Based on the data of the present study, glyphosate did not inhibit aromatase activity under the reported experimental conditions, and was therefore classified as a non-inhibitor of aromatase activity.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White crystalline solid
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93% glyphosate acid 85.14% calculated glyphosate content
Stability of test compound:	Not reported. Expiry: 2012-03-09
Vehicle, non-labelled substrate, and positive control:	
Solvent/vehicle used:	0.1 M sodium phosphate buffer (pH 7.4)
Non-radio labelled aromatase substrate:	4-Androstene-3,17-dione (ASDN) Source: Steraloids, Inc. Lot/Batch No.: L1712 Purity: 99.8% CAS No.: 63-05-8 Solvent used: Ethanol
Positive control (aromatase inhibitor):	4-hydroxyandrostendione (4-OH ASDN) Source: Sigma-Aldrich Lot/Batch No.: 081K2133 Purity: 99.6% CAS No.: 566-48-3 Solvent used: DMSO

Radiolabelled substrate:

Identification:	[1β-^{3}H]-Androstenedione ([^{3}H]ASDN)
Source:	Perkin-Elmer
Lot/Batch No.:	619344
Radiochemical purity:	>97%
Specific activity:	26.3 Ci/mmol, certification date 2010-08-06
Concentration of stock:	15 - 30 Ci/mmol
Solvent used:	Ethanol
Test system:	
Identification:	Human CYP19 (Aromatase) and P450 reductase Supersomes™ (BD Gentest™, BD Biosciences)
Lot/Batch No.:	19701
Protein concentration:	3.7 mg/mL

Cytochrome C reductase activity:	540 nmol cytochrome C reduced/mg protein/min
Aromatase activity:	5.7 pmol product/pmol P450/min
Test concentrations:	Dilutions of glyphosate and the positive control 4-OH ASDN were tested as shown in the table below. A full activity control (containing all test components plus solvent control) and a background activity control (containing all test components except NADPH plus solvent control) were also performed.

Table B.6.8.3.4-1: Glyphosate: Human Recombinant Aromatase Assay ([redacted] 2012): Competitor Final Molar (M) Concentrations in Aromatase Assay[1]

Positive control 4-OH ASDN	**Test substance Glyphosate**
1×10^{-5}	1×10^{-3}
1×10^{-6}	1×10^{-4}
$1 \times 10^{-6\ 5}$	1×10^{-5}
1×10^{-7}	1×10^{-6}
$1 \times 10^{-7\ 5}$	1×10^{-7}
1×10^{-8}	1×10^{-8}
1×10^{-9}	1×10^{-9}
1×10^{-10}	1×10^{-10}

[1] In each run of the assay, the full activity control and background activity control were run in replicates of four; the positive control was run in duplicate, and glyphosate was run in triplicate.

B. STUDY DESIGN AND TEST PERFORMANCE

Experimental dates: 2011-10-17 to 2011-10-21

Proficiency Testing
Proficiency testing of the CYP19 aromatase assay was conducted in three independent assay runs using the same methods as described below for the aromatase assay. Each run included evaluation of the positive control (4-OH ASDN) and the four recommended proficiency chemicals: econazole, fenarimol, nitrofen, and atrazine.

Aromatase Assay
Three types of control samples were included for each run. These included: 1) full enzyme (aromatase) activity controls (consisting of substrate [^{3}H]ASDN, NADPH, propylene glycol, buffer, microsomes, and vehicle [used for preparation of test substance solutions]); 2) background activity controls (all components that are in the full aromatase activity controls except NADPH); and 3) positive control (4-OH ASDN, run at eight concentrations, as indicated in the table above, in duplicate).

Four full activity controls and four background activity controls were included with each run of the assay (two tubes of each at the beginning of the assay and two tubes of each at the end of each assay).

Propylene glycol, [^{3}H] ASDN, NADPH, and assay buffer was combined in test tubes, with or without glyphosate or the positive control substance 4-OH-ASDN, for a total volume of 1 mL. The final concentrations for the major components of the assay are presented in the table below. The test tubes and microsomal suspensions were placed at 37 ± 2 °C in the water bath for five minutes prior to the initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume was 2 mL. Subsequently, the tubes were incubated for 15 minutes at 37 ± 2 °C. The reactions were terminated and the unreacted ASDN extracted. The amount of ^3H_2O in the aqueous fraction was quantified for each assay tube by liquid scintillation counting, and aromatase activity was reported in units of nmol/mg protein/min.

Table B.6.8.3.4-2: Glyphosate: Human Recombinant Aromatase Assay ([redacted], 2012): Summary of aromatase assay components and preparations

Assay Factor	Value
Sodium phosphate buffer (pH 7.4)	0.1 M
Microsomal protein	0.004 mg/mL[1]
NADPH	0.3 mM
[^{3}H]ASDN	100 nM
Propylene glycol	5%
Temperature	37 ± 2 °C
Incubation time	15 min

[1] The concentration of microsomal protein was optimized for microsomes that produce ~540 pmol product/mg protein/min and 5.7 pmol product/pmol P450/min.

C. DATA ANALYSIS

Statistics
The response curve was fitted by weighted non-linear regression analysis using a four-parameter logistic regression model. For each run, the individual percent of control values were plotted versus logarithm of the test chemical concentration. In order to determine the consistency among runs, the slope and LogIC_{50} for the positive control and test chemical(s) were compared across runs based on one-way random effects analysis of variance (ANOVA), with the runs treated as random effects. The parameters were graphed within each run with associated 95 % confidence intervals based on the within-run standard error, and the average across-run standard error, with the associated 95 % confidence interval incorporating run-to-run variation.

Classification of test material
The test material was classified based on the average of three valid runs of the assay. Each run was individually classified as follows:
Inhibitor = Average curve across runs crossed 50 %.
Non-Inhibitor = Average lowest portion of curve across runs is >75 % activity, or data do not fit the model.
Equivocal = Average lowest portion of curve across runs is between 50 % and 75 % activity.

II. RESULTS AND DISCUSSION

A. AROMATASE ASSAY
Mean aromatase activity in the full activity controls was 0.593 nmol/mg protein/min (range: 0.584 - 0.771 nmol/mg protein/min) for the three assay runs, which was greater than the minimum required aromatase activity of 0.1 nmol/mg protein/min according to guideline. The response of the full activity controls and background controls was acceptable for each run.

Aromatase assay results for glyphosate and the positive control 4-OH ASDN are shown in the table below and illustrated in the figure below.

For the positive control substance 4-OH ASDN, mean aromatase activity was 98.77 ± 1.54 % of vehicle control (VC) value at the lowest tested concentration of 10^{-10} M, and 0.67 ± 0.04 % of VC at the highest tested concentration of 10^{-5} M. Mean LogIC_{50} for 4-OH ASDN was -7.29 M (range: -7.28 to -7.30 M) and mean Hill slope was -0.96 (range: -0.92 to -1.00). Therefore, the aromatase activity results for the positive control substance were within the recommended ranges for the performance criteria.

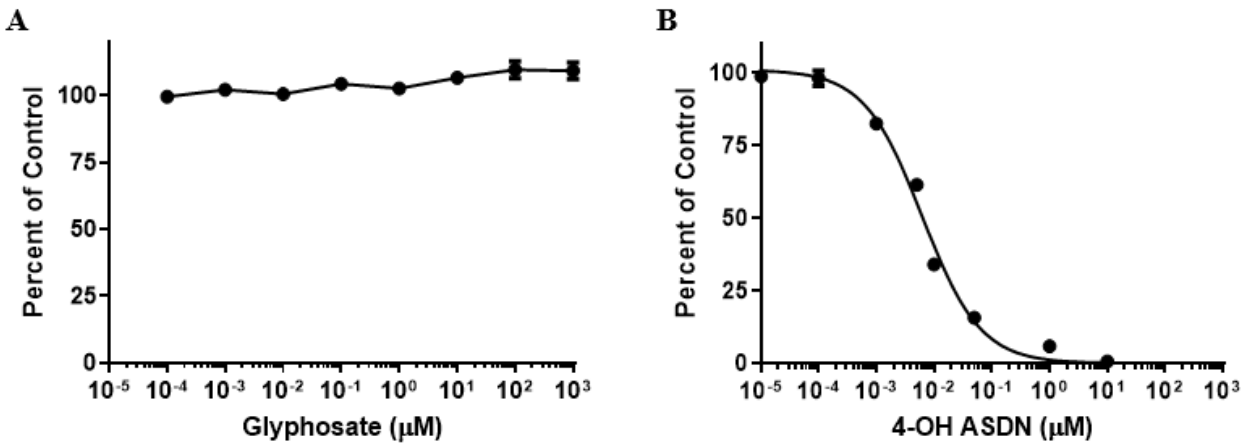
For the test substance glyphosate, mean aromatase activity was 99.67 ± 1.86 % of VC at the lowest tested concentration, 10^{-10} M and 109.3 ± 5.53 % of VC at the highest tested concentration, 10^{-3} M. LogIC_{50}. The Hill slope estimates were not determined for glyphosate because it never achieved >25 % inhibition. Based on these results, glyphosate does not inhibit the enzyme aromatase under the conditions of the present aromatase assay.

Table B.6.8.3.4-3: Glyphosate: Human Recombinant Aromatase Assay ([redacted], 2012): Effect of 4-OH ASDN and glyphosate on aromatase activity. Data are presented as mean activity in percent compared to control (mean ± SD).

Substance	Concentration (M)	Run 1	Run 2	Run 3	Overall Mean (% of control)
Positive control 4-OH ASDN	TA	99.90 ± 1.204	106.58 ± 0.255	103.18 ± 1.479	-
	NSB	0.02 ± 0.038	0.01 ± 0.042	0.02 ± 0.041	-
	10^{-5}	0.71 ± 0.011	0.64 ± 0.163	0.66 ± 0.047	0.67
	10^{-6}	6.38 ± 1.015	5.98 ± 0.727	5.49 ± 0.719	5.95
	$10^{-6\,5}$	16.86 ± 0.372	15.59 ± 0.104	14.73 ± 1.378	15.73
	10^{-7}	34.48 ± 0.218	34.06 ± 2.793	33.53 ± 4.675	34.02
	$10^{-7\,5}$	60.36 ± 3.002	61.35 ± 2.708	62.70 ± 0.854	61.47
	10^{-8}	83.62 ± 1.893	81.61 ± 3.938	82.45 ± 3.418	82.56
	10^{-9}	95.72 ± 4.292	97.76 ± 3.312	100.93 ± 1.042	98.14
	10^{-10}	97.99 ± 1.616	100.54 ± 2.450	97.77 ± 2.112	98.77
Test substance Glyphosate	TA	100.10 ± 2.217	93.42 ± 3.574	96.82 ± 0.708	-
	NSB	-0.02 ± 0.015	-0.01 ± 0.019	-0.02 ± 0.023	-
	10^{-3}	105.36 ± 2.440	106.92 ± 1.383	115.62 ± 0.313	109.30
	10^{-4}	107.19 ± 0.887	105.94 ± 5.294	116.07 ± 2.660	109.73
	10^{-5}	106.41 ± 1.959	105.22 ± 1.781	108.52 ± 0.341	106.72
	10^{-6}	103.64 ± 2.538	99.98 ± 4.675	104.63 ± 1.239	102.75
	10^{-7}	104.12 ± 2.743	103.66 ± 1.034	105.67 ± 0.671	104.48
	10^{-8}	100.27 ± 3.510	99.40 ± 6.362	102.33 ± 0.887	100.67
	10^{-9}	101.69 ± 2.054	102.86 ± 2.189	102.11 ± 2.912	102.22
	10^{-10}	100.84 ± 2.476	97.52 ± 3.393	100.64 ± 2.203	99.67

TA = Total activity, i.e. full activity control
NSB = Non-specific binding, i.e. background activity control

Figure B.6.8.3.4-1: Glyphosate: Human Recombinant Aromatase Assay ([redacted], 2012): Mean inhibition response curves for glyphosate (A) and 4-OH ASDN (B, positive control) (mean of three valid assays ± SEM)



III. CONCLUSIONS

Assessment and conclusion by applicant:
The study is performed in accordance with the current US OPPTS/OCSPP 890.1200 (2009). The study is therefore considered valid. In three independent runs of the present assay, increasing concentrations of

glyphosate showed no decrease in aromatase activity. The mean aromatase activity at all tested glyphosate concentrations was ≥ 99.67 % compared to control.
Based on these data, glyphosate did not inhibit aromatase activity under the reported experimental conditions, and was therefore classified as a non-inhibitor of aromatase activity.

Assessment and conclusion by RMS:

The conclusions made by the applicant are agreed with.

B.6.8.3.5. In vivo Uterotrophic assay

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats
Report No	[redacted]-843002
Document No	Not reported
Guidelines followed in study	US EPA OPPTS/OCSPP 890.1600 (2009) OECD 440 (2007)
Deviations from current test guideline (OCSPP 890.1600, 2009) (OECD 440, 2007)	None
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The characterization of the positive control substance, 17α-ethinyl estradiol, was not conducted according to GLP standards. However, the commercial provider characterized 17α-ethinyl estradiol and the certificate of analysis was included in the appendix of the final study report.
Acceptability/Reliability:	Conclusion GRG: Valid, category 2A Conclusion AGG: Study acceptable.

Executive summary
A uterotrophic assay was conducted in 66 - 67-day old female Crl:CD(SD) Sprague Dawley rats to screen for potential estrogenic activity of glyphosate. Groups of six ovariectomized rats each were administered glyphosate in 0.5 % methylcellulose (in deionized water) orally via daily gavage at dose levels of 0 (vehicle control), 100, 300, and 1000 mg/kg bw/day for three consecutive days. The high dose level represents the limit dose for the assay. A positive control group of six rats was administered a daily dose of 17α-ethinyl estradiol (EE) in corn oil at 3 µg/kg bw/day by subcutaneous (s.c.) injection. All rats were ovariectomized 17 - 18 days prior to substance administration.

All rats survived to scheduled necropsy 24 hours following the last substance administration. No test substance-related clinical findings were noted at the daily examinations or four hours following dose administration at any dose level. No clinical findings were noted in the positive control group. No macroscopic findings were noted in the uterus at 100, 300, or 1000 mg/kg bw/day glyphosate or in the positive control group.

No test substance-related effects on mean body weights or mean body weight gains were noted in glyphosate

treatment groups compared to concurrent control. Mean, absolute and relative, uterine weights (wet and blotted) of treatment groups were similar to control group values.
In positive control group animals, a mean body weight loss (-5.6 g) was observed at the end of the treatment period in contrast to a mean body weight gain (+11.3 g) in control group animals over the same period. Higher mean wet and blotted uterine weights (8.6- and 3.6-fold, respectively) were noted in the positive control group compared to control. These increases in uterine weights demonstrated the expected estrogenic effect of the positive control 17-α-ethinyl estradiol.

Based on the lack of effects on mean uterine weights (wet and blotted), glyphosate did not demonstrate or mimic biological activities consistent with agonism of natural estrogens when administered orally to ovariectomized female rats at dosage levels of 100, 300, and 1000 mg/kg bw/day. The positive control substance (17α-ethynylestradiol) elicited the expected increases in wet and blotted uterine weights.

The absence of effects on uterine weights demonstrated a lack of estrogenicity for glyphosate at all dosage levels evaluated up to and including the limit dose of 1000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White powder
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93% glyphosate acid 85.14% calculated glyphosate content
Stability of test compound:	Stored at room temperature and considered stable under this condition.

Vehicle and positive control:

Solvent/vehicle used:	Methylcellulose Source: Sigma-Aldrich Lot/Batch No.: 060M0123V CAS No.: 9004-67-5 Final concentration: 0.5% in deionized water
Positive Control (reference estrogen):	17α-ethinyl estradiol (EE) Source: Sigma-Aldrich Lot/Batch No.: 028K1411 Purity: 99% CAS No.: 57-63-6 Solvent used: corn oil

Test animals:

Species:	Rat
Strain:	Crl:CD(SD) Sprague Dawley
Source:	[redacted]
Age at ovariectomy:	49 days of age; ovariectomy was performed at [redacted]
Age at dosing:	66 - 67 days of age; vaginal lavages were performed daily for five consecutive days (beginning at least 12 days after ovariectomy) prior to assignment to study groups, to ensure females were in persistent diestrus
Sex:	Female
Weight at dosing:	245.6 – 301.2 g
Acclimation period:	11 days

Diet/ Food:	2016*CM* Teklad Global 16 % Protein Rodent Diet (mean total isoflavones: 29.0 ppm), *ad libitum*	
Water:	Reverse osmosis-purified tap water, *ad libitum*	
Housing:	Individually housed in clean, stainless steel wire-mesh cages suspended above cage-board	
Environmental conditions:	Temperature:	21.3 - 21.6 °C
	Humidity:	51.8 - 55.2 %
	Air changes:	10/hour
	Photocycle:	12 hours light/ 12 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 02 Jul to 04 Jul 2011 test substance administration; 05 Jul 2011 necropsy

Animal assignment and treatment
Animals were assigned to groups using a WTDMS™ computer program that randomised the animals based on stratification of body weights in a block design. The experimental design consisted of one vehicle control group, three test substance-treated groups, and one positive control group, each composed of six rats.

The vehicle and test substance formulations were administered orally by gavage once daily during study days 0 - 2. The oral dosage volume for all groups was 5 mL/kg bw. The positive control substance, 17α-ethinyl estradiol, was administered by bolus subcutaneous (s.c.) injection in the scapular region at a dosage volume of 5 mL/kg bw. Dosing sites were rotated clockwise between three distinct scapular sites daily. Individual dosages were based on the most recently recorded body weights.

Table B.6.8.3.5-1: A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats ([redacted], 2012): Study design of uterotrophic assay with glyphosate, administered orally to female Sprague Dawley rats

Test group	Dose (mg/kg bw/day)[1]	No. of females
Control (vehicle)[2]	0	6
Low dose[2]	100	6
Mid dose[2]	300	6
High dose[2]	1000	6
Positive control 17α-ethinyl estradiol (EE)[3]	0.003	6

[1] Dosing volume: 5 mL/kg bw; a correction factor of 85.14 % was used to account for glyphosate purity.
[2] Exposure route: oral (gavage).
[3] Exposure route: subcutaneous (injection).

Dose Preparation and analysis
The test substance formulations were prepared once as single formulations for each dose level, divided into aliquots for daily dispensing, and stored at room temperature. A stock solution of 17α-ethinyl estradiol (0.05 mg/mL) in corn oil was prepared one day prior to the initiation of dosing. The positive control formulation was prepared by serial dilution of the stock solution in corn oil. The test substance and positive control formulations were prepared at separate stations to avoid cross contamination and were stirred continuously throughout the preparation, sampling and dose administration procedures. Samples for concentration analysis were collected from the middle stratum of each dosing formulation (including the positive control group) prepared during the in-life phase of the study. Homogeneity, resuspension homogeneity, and stability of the test substance formulations were demonstrated in a preceding, separate study.

Observations
All rats were observed (cage-side) twice daily for clinical signs of toxicity, moribund condition and mortality. Individual clinical observations (hand-held physical examination) were recorded daily (prior to dose administration during the treatment period) through the day of necropsy. Animals were also observed for signs of toxicity approximately four hours following dose administration. Animals of the positive control group were examined for potential erythema, swelling, or other dermal findings at the injection site.

Body weight
Individual body weights were recorded on the day of randomization, daily prior to test substance administration, and on the day of euthanasia. Mean body weight changes were calculated for each corresponding interval and for study days 0 - 3.

Food consumption
Food consumption was not evaluated.

Sacrifice and measurement of uterine weight
All females were euthanized by carbon dioxide inhalation approximately 24 hours following administration of the last dose in the same order of dosing. Macroscopic examination was conducted for the uteri; full necropsies were not conducted. The uterus was carefully dissected and trimmed, being careful to retain the luminal fluid. Each "wet" uterus was weighed intact (with the luminal fluid) to the nearest 0.1 mg, then opened longitudinally and blotted with filter paper to remove the luminal fluid. Subsequently, the blotted uterus was weighed to the nearest 0.1 mg. Any loss in luminal content was reported.

Microscopic Examination
Microscopic examinations were not conducted. However, the uterus and vagina were preserved in 10% neutral-buffered formalin for possible future histopathologic examination.

Statistics
Each endpoint was tested for homogeneity of variance using Levene's test. Homogeneity of variance was confirmed for each endpoint ($p > 0.01$) and no data transformation was required.

Uterine weights: In the first stage, the 17α-ethinyl estradiol group was compared to the vehicle control group. An analysis of covariance (ANCOVA) was performed using the body weight at necropsy as the covariate. Both groups were compared to each other in a one-sided test using $p = 0.05$ to declare significance, looking for significant increases from the vehicle control.

The second stage of the analysis compared the various glyphosate-treated groups to the vehicle control group. An ANCOVA was performed using the body weight at necropsy as the covariate. Comparisons of each treatment group with the vehicle control group were made with a one-sided Dunnett's test using $p = 0.05$ to declare significance, looking for significant increases from the vehicle control group.

Body weights and cumulative body weight change: The statistical analysis of body weight and body weight change was identical to the uterine weights with the following exception: there was no covariate in the statistical model (ANOVA) and the testing was two-sided rather than one-sided.

II. RESULTS AND DISCUSSION

The analysed dosing formulation were within the acceptable range (85-115%).

A. MORTALITY
All animals survived until scheduled necropsy.

B. CLINICAL OBSERVATIONS
No test substance-related clinical findings were noted at the daily examinations at any dose level. No clinical findings were noted four hours following dose administration at any dose level. No clinical observations were noted in the positive control group. Slight and/or very slight oedemas were noted at the injection sites of positive control animals.

C. BODY WEIGHT
No test substance-related effects on mean body weights or body weight changes were noted in the 100, 300, and 1000 mg/kg bw/day glyphosate-treated groups. The values in the test substance-treated groups were similar to and not statistically significantly different from the vehicle control group.

In the positive control group, a significant ($p < 0.0001$) mean body weight loss (-5.6 g) was noted when the

overall treatment period (study days 0 - 3) was evaluated compared to a mean body weight gain in the vehicle control group (+11.3 g). This resulted in a decreased mean terminal body weight in the positive control group (-5.24%) compared to the vehicle control group on study day 3. The difference was not statistically significant. Body weights and body weight changes are presented in the table below.

Table B.6.8.3.5-2: A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats ([redacted], 2012): Mean group body weight and body weight change in the uterotrophic assay in Sprague Dawley rats. Females were exposed to glyphosate for three days via gavage (n=6).

Dose (mg/kg bw/day)	Body weight (g), mean ± SD				Body weight change (g)
	Study Day 0	Study Day 1	Study Day 2	Study Day 3	
0	277.0 ± 19.18	279.7 ± 19.50	286.4 ± 21.12	288.3 ± 20.96	11.3
100	277.6 ± 14.63	282.4 ± 15.81	288.2 ± 16.34	292.9 ± 15.98	15.3
300	275.2 ± 15.61	279.2 ± 16.88	284.8 ± 15.54	284.3 ± 19.40	9.1
1000	278.3 ± 15.48	282.8 ± 16.64	287.5 ± 16.50	291.6 ± 17.53	13.3
0.003 EE	278.7 ± 18.79	280.2 ± 21.06	278.2 ± 19.36	273.2 ± 20.92	-5.6

SD, standard deviation; EE, 17α-ethinyl estradiol (positive control)

D. NECROPSY

At scheduled necropsy, no macroscopic findings in the uterus were observed at dosage levels of 100, 300, and 1000 mg/kg bw/day glyphosate or in the positive control group.

E. UTERINE WEIGHTS

Mean wet and blotted uterine weights were unaffected by test substance administration at dose levels of 100, 300, and 1000 mg/kg bw/day. Differences from the vehicle control group were slight and not statistically significant. Mean blotted uterine weight in the vehicle control group was less than 0.04% of the final body weight, indicating acceptable results.

The positive control substance produced the expected estrogenic response. Mean wet and blotted uterine weights in this group were significantly ($p < 0.0001$) higher (8.6- and 3.6-fold, respectively) than the vehicle control group values. Results of wet and blotted uterine weights are presented in the table below.

Table B.6.8.3.5-3: A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats ([redacted], 2012): Uterine weights in the uterotrophic assay Sprague Dawley rats treated orally with glyphosate for three days.

Dose (mg/kg bw/day)	0	100	300	1000	0.003 EE
Parameter	**Uterine weight (mean ± SD)**				
Terminal body weight	289 ± 20.9	293 ± 16.2	284 ± 19.1	292 ± 17.5	273 ± 21.0
Wet, absolute (mg)	111.0 ± 10.80	110.7 ± 12.45	118.3 ± 16.49	113.6 ± 9.66	953.1* ± 90.44
Wet, relative (%)	0.038 ± 0.0025	0.038 ± 0.0056	0.042 ± 0.0061	0.039 ± 0.0044	0.352* ± 0.0546
Blotted, absolute (mg)	98.2 ± 11.66	98.7 ± 10.62	103.0 ± 11.62	102.4 ± 8.87	349.3* ± 31.14
Blotted, relative (%)	0.034 ± 0.0024	0.034 ± 0.0048	0.036 ± 0.0040	0.035 ± 0.0038	0.129* ± 0.0173

SD, standard deviation; EE, 17α-ethinyl estradiol (positive control); * Significantly different from control at $p < 0.0001$

III. CONCLUSIONS

Assessment and conclusion by applicant:

The study is performed in accordance with the current US EPA OPPTS/OCSPP 890.1600 (2009) and OECD 440 (2007). The study is therefore considered valid. Oral administration of glyphosate at dosage levels up to 1000 mg/kg bw/day did not produce any indication of toxicity. In addition, mean uterine weights (wet and blotted) in the glyphosate-treated groups were similar to control group values. The absence of effects on uterine weights

demonstrated a lack of estrogenicity for glyphosate at all dose levels evaluated up to and including the limit dose of 1000 mg/kg bw/day in the present uterotrophic assay. The positive control 17α-ethinyl estradiol elicited expected, significant increase in absolute wet and blotted uterine weights compared to the control group, demonstrating the sensitivity of the assay.
Based on these results, glyphosate did not demonstrate or mimic biological activities consistent with agonism of natural estrogens at all applied doses including the limit dose of 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

The study is considered to be acceptable.

Based on the results of the study glyphosate did not demonstrate an estrogenic effect.
This conclusion is in line with the previous EU evaluation (addendum 2 to the RAR, 2017).

B.6.8.3.6. In vivo Hershberger assay

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats
Report No	[redacted]-843003
Document No	Not reported
Guidelines followed in study	US EPA OPPTS/OCSPP 890.1400 (2009) OECD 441 (2009)
Deviations from current test guideline (OCSPP 890.1400, 2009) (OECD 441, 2009)	None
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The characterisation of the positive control substances, testosterone propionate and flutamide, was not conducted according to GLP standards but were characterized by the commercial provider and documented on the certificate of analysis.
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: Acceptable.

Executive summary
The objective of this study was a mechanistic *in vivo* screen for potential androgenic activity of the test substance glyphosate. To screen for potential androgenic activity, glyphosate (in 0.5% methylcellulose in deionized water) was administered daily for ten days via gavage to 54 - 55-day old, castrated male Crl:CD(SD) Sprague Dawley rats (six per dose group) at dose levels of 0 (vehicle control), 100, 300, or 1000 mg/kg bw/day. The high dose represents the limit dose for this assay. An androgenic positive control group consisted of six castrated rats exposed to 0.2 mg/kg bw/day testosterone propionate (TP) by subcutaneous (s.c.) injection. To screen for potential anti-androgenic activity, glyphosate in 0.5% methylcellulose (in deionized water) was administered daily for ten days via gavage to 54 - 55-day old, castrated male Crl:CD(SD) Sprague Dawley rats (six per dose group) at dose levels of 0 (vehicle control), 100, 300, or 1000 mg/kg bw/day, in conjunction with a

daily dose of the reference androgen TP at 0.2 mg/kg bw/day by s.c. injection. An anti-androgenic positive control group consisted of six castrated rats exposed to 0.2 mg/kg bw/day TP by s.c injection, and to 3 mg/kg bw/day flutamide (FT) by oral gavage. TP alone was used as the anti-androgenic negative control.

All animals were observed twice daily for mortality and moribundity. Clinical observations and body weights were recorded daily. The bulbourethral glands, glans penis, levator ani and bulbocavernosus (LABC) muscle group, seminal vesicles with coagulating glands, and ventral prostate were examined, weighed, and retained in 10 % neutral-buffered formalin.

All males survived to scheduled euthanasia on study day 10. There were no test substance-related clinical or macroscopic findings noted at any dose level. No test substance or control substance-related effects on mean body weights or body weight change were noted. No test substance-related androgenic effects on the weights of male accessory sex organs (bulbourethral glands, glans penis, LABC muscle group, seminal vesicles with coagulating glands, and ventral prostate) were noted compared to the vehicle control group. Co-administration of TP with the test substance did not reveal an anti-androgenic effect (i.e. decreased mean organ weights) when compared to the TP control group. Administration of the androgenic positive control substance (TP) resulted in the expected higher mean male accessory sex organ weights. Co-administration of the anti-androgenic positive control substance flutamide (FT) with TP inhibited the androgenic response, resulting in lower mean organ weights compared to the TP control group.

Based on the results of the present Hershberger assay, glyphosate was negative for androgenicity and anti-androgenicity under the reported conditions.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White powder
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93% glyphosate acid 85.14% calculated glyphosate content
Stability of test compound:	Stored at room temperature and was considered stable under this condition
Vehicle and positive controls:	
Vehicle:	Methylcellulose Source: Sigma-Aldrich Lot/Batch No.: 060M0123V CAS No.: 9004-67-5 Final concentration: 0.5% in deionized water
Positive Control (reference androgen):	Testosterone propionate (TP) Source: AK Scientific, Inc., Mountain View, CA, US Lot/Batch No.: 70321J Purity: 98.3% CAS No.: 57-82-5 Solvent used: minimal amount of 95% ethanol Vehicle used: corn oil

Positive Control (reference anti-androgen):	Flutamide (FT) Source: Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, US Lot/Batch No.: 2AC0144 Purity: 100% CAS No.: 1311-84-7 Solvent used: minimal amount of 95 % ethanol Vehicle used: corn oil
Test animals:	
Species:	Rat
Strain:	Crl:CD(SD) Sprague Dawley
Source:	[redacted]
Age at castration:	42 days of age; orchidoepididymectomy was performed at [redacted]
Age at start of dosing:	54 - 55 days of age
Sex:	Male
Weight at dosing:	211.3 – 279.2 g
Acclimation period:	13 days post-castration, 6 days post-arrival at laboratory
Diet/Food:	PMI Nutrition International, LLC Certified Rodent LabDiet 5002, *ad libitum*
Water:	Municipal water filtered by reverse-osmosis, *ad libitum*
Housing:	Individually housed in clean, stainless steel wire-mesh cages suspended above cage-board
Environmental conditions:	Temperature: 21.3 - 21.6 ºC Humidity: 51.8 - 55.2% Air changes: 10/h Photocycle: 12 hours light/ 12 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 27 Jun to 06 Jul 2011 test substance administration; 07 Jul 2011 necropsy

Animal assignment and treatment
Males were assigned to groups using a WTDMS™ computer program which randomized the animals based on body weight stratification in a block design. The experimental design consisted of two assays: one for androgen agonist activity and one for anti-androgen activity. In the androgen agonist assay, there was one vehicle control group, one positive control group and three test substance-treated groups, each composed of six rats. In the anti-androgen assay, there was one vehicle control group, one androgen treated negative control group, one androgen and anti-androgen treated positive control group, and three test substance-treated groups (co-treated with the androgen TP), each composed of six rats.

In the Hershberger assay to screen for potential androgenic activity, the test substance glyphosate was administered daily for ten days via oral gavage to castrated male rats at doses of 100, 300, or 1000 mg/kg bw/day (at a dosing volume of 5 mL/kg bw).

In the Hershberger assay to screen for potential anti- androgenic activity, glyphosate was administered daily for ten days via gavage to castrated male rats at the same doses (100, 300, or 1000 mg/kg bw/day) in conjunction with a daily dose of TP (0.2 mg/kg bw/day) by s.c. injection.

A concurrent vehicle control group received the dosing vehicle by gavage for the same duration. In another group, TP was administered via s.c. injection at a dose level of 0.2 mg/kg bw/day and a dosing volume of 0.5 mg/mL. This treatment served as an androgenic positive control group in the androgen agonist assay as well as an anti-androgen negative control in the anti-androgen assay (same animals). In the anti-androgen positive control group, males received 0.2 mg/kg bw/day TP by s.c. injection in conjunction with 3 mg/kg bw/day of flutamide (FT), a known anti-androgenic substance, by oral gavage (at a dosing volume of 5 mL/kg bw) for ten

days.

Table B.6.8.3.6-1: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats ([redacted], 2012): Study design of Hershberger assay with glyphosate in male Sprague Dawley rats

Test group	Dose[1] (mg/kg bw/day)	No. of males	Dosing volume (mL/kg bw/day)
Androgen agonist assay			
Control (vehicle)	0	6	5
Low	100	6	5
Mid	300	6	5
High	1000	6	5
Positive Control (TP, s.c.)	0.2	6	0.5
Anti-androgen assay			
Control (vehicle)	0	6	5
Negative Control (TP, s.c.)	0.2	6	0.5
Low (+ TP, s.c.)	100 (+ 0.2)	6	5 (+ 0.5)
Mid (+ TP, s.c.)	300 (+ 0.2)	6	5 (+ 0.5)
High (+ TP, s.c.)	1000 (+ 0.2)	6	5 (+ 0.5)
Positive Control (FT, gavage) (+ TP, s.c.)	3 (+ 0.2)	6	5 (+ 0.5)

s.c. = subcutaneous; TP = Testosterone propionate; FT = Flutamide

[1] To calculate the glyphosate dose a correction factor of 85.14 % was used to account for the purity.

Dose Preparation and Analysis

The test substance formulations were prepared approximately weekly as single formulations for each dose level, divided into aliquots for daily dispensation, and stored at room temperature. The test substance formulations were stirred continuously throughout the preparation, sampling, and dose administration procedures.

The positive control substance formulations were prepared once, as follows. The appropriate amount of the positive control substance (TP or FT) was dissolved in a minimal amount of 95% ethanol and diluted to the final concentration with corn oil. The FT formulation was then divided into aliquots for daily use. Aliquots of the TP formulation were dispensed into sterile septum vials for daily use. The positive control substance formulations were stored at room temperature and were stirred continuously throughout the preparation, sampling, and dose administration procedures.

The vehicle was prepared approximately weekly for administration to the vehicle control group and for preparation of the test substance formulations; aliquots were prepared for daily dispensation to the control group and stored at room temperature. The vehicle was mixed throughout the preparation, sampling, and dose administration procedures.

Clinical observations

All rats were observed (cage-side) twice daily for moribund condition and mortality. Individual clinical observations (hand-held physical examinations) were recorded daily (prior to test substance administration during the treatment period) through the day of scheduled necropsy. Each male was also observed for signs of toxicity at approximately four hours following dose administration. The injection sites for males receiving s.c. injections were examined daily from the initiation of dose administration for erythema, swelling, and other dermal findings. Erythema and swelling were evaluated in accordance with a four-step grading system of very slight, slight, moderate, and severe.

Body weight

Individual male body weights were recorded on the day of randomization, daily prior to test substance administration, and on the day of scheduled necropsy. Mean body weight changes were calculated for each corresponding interval and for study days 0 - 10.

Food consumption

Food consumption was not recorded.

Blood collection
Blood samples (3 mL/animal) were collected from the vena cava following anaesthesia by isoflurane inhalation approximately 24 hours following administration of the last dose. The blood samples were allowed to clot at room temperature, serum was separated by centrifugation, and the samples stored frozen (approximately -70 °C) for possible future hormone analysis.

Sacrifice, measurement of accessory sex organ weights and pathology
Males were euthanized by exsanguination approximately 24 hours following the last administration. Full necropsies were not conducted. The following tissues and organs were excised, trimmed free of adhering tissue, and weighed fresh (unfixed) and placed in 10 % neutral-buffered formalin:

- Bulbourethral glands (Cowper's gland)[1, 2]
- Glans penis
- Levator ani and bulbocavernosus (LABC) muscle group
- Seminal vesicles with coagulating glands[1, 2]
- Ventral prostate[2]

[1] = Paired organs were weighed together.
[2] = Care was taken to minimize fluid loss from these organs; loss of fluid was documented.

Microscopic examinations were not conducted.

Statistics
Each endpoint was tested for homogeneity of variance using Levene's test. If that test was significant at p = 0.01, then a log transformation was applied and Levene's test conducted on the transformed data. Homogeneity of variance was confirmed for each endpoint (p >0.01).

Androgen agonist assay:
Organ weights (vehicle control vs. TP): The one-sided t-test was performed using p = 0.05 to declare significance, looking for significant increases from the vehicle control group.
Organ weights (vehicle control vs. glyphosate groups): An analysis of variance (ANOVA) was conducted on the raw or log transformed data. A one-sided Dunnett's test was conducted using p = 0.05 to declare significance, regardless of the outcome of the ANOVA, looking for significant increases in the glyphosate groups when compared with the vehicle control group.
Body weight and body weight change: The statistical analysis was identical to that for organ weights with the exception that the testing was two-sided, rather than one-sided, using p = 0.05 to declare significance.

Anti-androgen assay:
Organ weights (TP vs. TP + FT): A one-sided t-test was performed using p = 0.05 to declare significance, looking for significant decreases from the TP group.
Organ weights (TP vs. glyphosate + TP groups): An ANOVA was conducted on the raw or transformed data, as appropriate. A one-sided Dunnett's test was conducted using p = 0.05 to declare significance, regardless of the outcome of the ANOVA, looking for significant decreases in the glyphosate + TP groups when compared with the TP group.
Body weight and body weight change: The statistical analysis was identical to the organ weights with the exception that the testing was two-sided, rather than one-sided, using p = 0.05 to declare significance.

Performance criteria
Because the organ weights of different strains of rats vary, absolute organ weights are not used as the performance criteria in the Hershberger assay. Rather, the performance criteria are based on the coefficient of variance (CV) for each organ. For negative outcomes, the CVs from the control and high dose group were examined to determine if the maximum CV performance was exceeded. To meet the performance criteria, the maximum allowable CV for each organ as set out in the test guideline (OECD 441) was applied.

II. RESULTS AND DISCUSSION

The analysed doses were within the acceptable range (85-115%).

A. MORTALITY
There were no mortalities. All animals survived until scheduled necropsy.

B. CLINICAL OBSERVATIONS
No test substance- or positive control-related clinical findings were noted. Single occurrences of very slight erythema, very slight edema, and slight edema were noted in animals receiving s.c. injection of TP and TP/FT. One animal with desquamation was noted in the TP positive control group.

C. BODY WEIGHT
No test substance-related effects on mean body weights or body weight changes were noted in the 100, 300, and 1000 mg/kg bw/day glyphosate-treated groups compared to the control group (see tables below). In the test substance-treated groups co-administered TP, mean body weights and body weight changes were similar to those of the TP control group. Mean body weights and body weight changes in the TP/FT group were similar to those of the TP control group throughout the study. In the TP control group, mean body weights and body weight changes were similar to those of the vehicle control group. Altogether, no statistically significant differences were noted.

Table B.6.8.3.6-2: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats ([redacted], 2012): Body weight (bw) and cumulative body weight change in the androgen agonist assay in Sprague Dawley rats administered glyphosate via oral gavage for ten days

Body weight (g)										
Study group	**Control**		**Positive control TP**		**100 mg/kg bw/day**		**300 mg/kg bw/day**		**1000 mg/kg bw/day**	
Study day	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
0	243.3	14.70	237.7	18.38	240.9	15.72	240.9	13.47	240.9	13.68
1	248.6	15.10	243.5	17.80	245.8	17.41	246.8	16.05	246.1	12.17
2	256.4	14.70	249.3	19.68	252.3	17.10	252.4	15.44	253.1	12.85
3	261.7	14.28	257.0	21.42	258.5	17.32	259.5	17.21	258.2	13.20
4	268.5	14.15	265.5	21.58	265.9	16.03	267.0	17.55	266.9	12.10
5	268.8	13.88	265.9	23.19	266.1	16.54	266.7	18.91	260.4	20.32
6	281.2	13.98	278.2	23.62	276.7	17.63	279.3	20.24	271.7	23.53
7	287.0	13.76	286.1	24.08	283.0	16.89	286.0	19.20	276.3	22.36
8	295.8	14.48	296.7	26.22	293.3	17.17	295.8	21.61	286.5	23.75
9	294.6	12.63	295.4	27.00	292.2	17.19	294.7	19.26	286.0	20.90
10	309.1	13.22	311.1	31.77	306.6	17.48	308.8	23.15	300.5	20.94
BW change Study day 0 - 10	**+65.8**		**+73.4**		**+65.7**		**+67.9**		**+59.6**	

SD = standard deviation; TP = Testosterone propionate; n = 6 animals/group

Table B.6.8.3.6-3: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats ([redacted], 2012): Body weight and cumulative body weight (bw) change in the anti-androgen assay in Sprague Dawley rats administered glyphosate via oral gavage for ten days

Body weight (g)												
Study group	**Control**		**Negative Control TP**		**Positive Control FT + TP**		**100 mg/kg bw/day + TP**		**300 mg/kg bw/day + TP**		**1000 mg/kg bw/day + TP**	
Study day	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
0	243.3	14.70	237.7	18.38	240.6	17.78	238.3	16.13	239.6	16.54	242.1	20.44

Body weight (g)												
Study group	**Control**		**Negative Control TP**		**Positive Control FT + TP**		**100 mg/kg bw/day + TP**		**300 mg/kg bw/day + TP**		**1000 mg/kg bw/day + TP**	
Study day	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
1	248.6	15.10	243.5	17.80	247.1	18.52	242.3	18.77	248.4	16.91	249.1	19.50
2	256.4	14.70	249.3	19.68	254.1	20.91	250.0	17.74	255.7	17.16	255.6	22.65
3	261.7	14.28	257.0	21.42	262.0	21.14	257.6	16.80	264.9	17.32	265.2	22.70
4	268.5	14.15	265.5	21.58	270.1	23.87	268.2	17.12	274.3	17.85	272.8	22.00
5	268.8	13.88	265.9	23.19	270.3	24.18	268.7	16.59	274.1	18.95	274.4	25.20
6	281.2	13.98	278.2	23.62	281.8	26.67	281.6	17.82	286.5	17.67	286.3	24.10
7	287.0	13.76	286.1	24.08	288.1	25.12	289.7	16.54	295.4	18.16	293.0	22.30
8	295.8	14.48	296.7	26.22	297.8	27.92	302.2	18.63	307.6	17.47	305.3	23.21
9	294.6	12.63	295.4	27.00	296.9	26.89	301.7	17.72	306.0	17.50	305.6	26.43
10	309.1	13.22	311.1	31.77	310.6	28.49	317.2	16.66	322.4	19.47	320.1	26.51
BW change Study day 0 - 10	**65.8**		**73.4**		**70.0**		**78.9**		**82.8**		**78.0**	

TP = Testosterone propionate; FT = Flutamide; SD = standard deviation; n = 6 animals/group

D. ACCESSORY SEX ORGAN WEIGHTS

No evidence of androgenic agonism was noted in the 100, 300, or 1000 mg/kg bw/day glyphosate treated groups based on the lack of higher mean weights of the bulbourethral gland, glans penis, LABC muscle group, seminal vesicles/coagulating gland, and ventral prostate (see Table B.6.8.3.6-4). Differences from the vehicle control group were slight and were not statistically significant.

No evidence of androgenic antagonism (anti-androgenic activity) was noted in the 100, 300, or 1000 mg/kg bw/day glyphosate treated groups co-administered TP (see Table B.6.8.3.6-4). Slight differences of glyphosate +TP dose groups from the TP control group were not statistically significant.

The androgenic positive control substance (concurrently the anti-androgenic negative control) TP elicited the expected response; statistically significant higher mean bulbourethral gland ($p < 0.0001$), glans penis ($p = 0.0007$), LABC muscle group ($p < 0.0001$), seminal vesicles/coagulating gland ($p < 0.0001$), and ventral prostate ($p < 0.0001$) weights compared to the vehicle control group. The anti-androgenic positive control FT inhibited the androgenic effect of co-administered TP, resulting in statistically significantly ($p \leq 0.0001$) decreased mean bulbourethral gland, glans penis, LABC muscle group, seminal vesicles/coagulating gland, and ventral prostate weights compared to the TP control group. All CV values were within the range of the assay's performance criteria.

Table B.6.8.3.6-4: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats ([redacted], 2012): Accessory sex organ weights of the androgen agonist assay in Sprague Dawley rats administered glyphosate via oral gavage for ten days

Organ	**Dose (mg/kg bw /day)**					
	Control			**Positive control TP (0.2)**		
	Mean (mg)	**± SD**	**CV**	**Mean (mg)**	**± SD**	**CV**
Bulbourethral	6.2	1.33	21.53	28.6**	6.35	22.17
Glans penis	105.0	15.92	15.16	152.1**	20.85	13.71
LABC	157.3	22.02	14.00	471.6**	63.13	13.39

Seminal vesicles with coagulating glands	86.4		12.06	13.96		463.6**	35.93		7.75
Ventral prostate	16.2		3.73	23.00		134.2**	22.20		16.54
Organ	**Dose (mg/kg bw /day)**								
	100			**300**			**1000**		
	Mean (mg)	**± SD**	**CV**	**Mean (mg)**	**± SD**	**CV**	**Mean (mg)**	**± SD**	**CV**
Bulbourethral	6.6	2.00	30.39	7.0	2.01	28.76	6.4	1.46	22.99
Glans penis	102.0	14.36	14.08	103.6	20.14	19.44	100.8	16.36	16.22
LABC	160.0	30.47	19.04	151.8	14.23	9.38	164.3	19.30	11.75
Seminal vesicles with coagulating glands	89.3	14.03	15.72	93.1	14.66	15.76	84.4	19.34	22.91
Ventral prostate	21.5	9.39	43.74	15.1	5.49	36.45	17.2	4.26	24.79

TP = Testosterone propionate; SD = Standard deviation; CV = Coefficient of variation; LABC = Levator ani and bulbocavernosus; ** = Significantly different from controls at p <0.01; n = 6 animals/ group

Table B.6.8.3.6-5: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats ([redacted], 2012): Accessory sex organ weights of the anti-androgen assay in Sprague Dawley rats administered glyphosate via oral gavage for ten days

Organ	**Dose (mg/kg bw /day)**								
	Control			**Negative control TP (0.2)**			**Positive control FT + TP**		
	Mean (mg)	**± SD**	**CV**	**Mean (mg)**	**± SD**	**CV**	**Mean (mg)**	**± SD**	**CV**
Bulbourethral	6.2	1.33	21.53	28.6**	6.35	22.17	8.7**	1.92	22.10
Glans penis	105.0	15.92	15.16	152.1*	20.85	13.71	108.3*	5.89	5.44
LABC	157.3	22.02	14.00	471.6*	63.13	13.39	173.9*	31.33	18.02
Seminal vesicles with coagulating glands	86.4	12.06	13.96	463.6**	35.93	7.75	111.9**	17.50	15.63
Ventral prostate	16.2	3.73	23.00	134.2*	22.20	16.54	27.0**	8.18	30.31
Organ	**Dose (mg/kg bw /day)**								
	100 + TP			**300 + TP**			**1000 + TP**		
	Mean (mg)	**± SD**	**CV**	**Mean (mg)**	**± SD**	**CV**	**Mean (mg)**	**± SD**	**CV**
Bulbourethral	31.1	3.12	10.03	28.5	9.22	32.40	29.9	4.95	16.57
Glans penis	150.6	17.86	11.86	148.8	23.69	15.92	168.2	15.42	9.17
LABC	405.4	49.04	12.10	461.0	92.12	19.98	471.8	83.07	17.61
Seminal vesicles with coagulating glands	411.0	71.92	17.50	417.5	99.47	23.82	391.6	75.25	19.22
Ventral prostate	112.2	18.51	16.50	110.6	26.48	23.95	125.3	25.30	20.19

TP = Testosterone propionate; FT = Flutamide; SD = Standard Deviation; CV = Coefficient of Variation; LABC = Levator ani and bulbocavernosus; ** = Significantly different from controls at p <0.01; n = 6 animals/ group

III. CONCLUSION

Assessment and conclusion by applicant:
The study is performed in accordance with the current US EPA OPPTS/OCSPP 890.1400 (2009) and OECD 441 (2009). The study is therefore considered valid. Glyphosate did not have androgenic or anti-androgenic activity in the Hershberger assay at dose levels of 100, 300, or 1000 mg/kg bw/day. In the androgen agonist assay, the positive control TP elicited the appropriate responses of increased androgen-dependent organ weights. In the anti-androgen assay, the positive control FT inhibited TP induced increases in androgen-dependent organ weights when compared to the TP control group. Organ weights of glyphosate treated animals were not affected when compared to the appropriate control group. Glyphosate alone dose groups were not androgenic compared with the vehicle control group. Glyphosate + TP dose groups were not anti-androgenic compared with the TP alone anti-androgenic negative control group. Based on these results, glyphosate was negative for androgenicity and anti-androgenicity in the Hershberger assay.

Assessment and conclusion by RMS:

The study is considered to be acceptable.

Based on the results of the study glyphosate did not demonstrate an androgenic or anti-androgenic effect.
This conclusion is in line with the previous evaluation (addendum 2 to the RAR, 2017).

B.6.8.3.7. In vivo Pubertal assay in male rats

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats
Report No	[redacted]-843005
Document No	Not reported
Guidelines followed in study	US EPA OPPTS/OCSPP 890.1500 (2009)
Deviations from current test guideline (OCSPP 890.1500, 2009)	Some values of the control group were outside the guideline performance criteria range (mean body weight at time of weaning (PND 21), CV value for body weight at attainment of PPS, CV value for mean TSH level, and mean thyroid gland and kidney weights). However, CV values of mean body weight (PND 21) and thyroid and kidney weights; mean body weight at the attainment of PPS and the mean/CV value for the age of attainment of PPS; and mean TSH value were in the acceptable guideline performance criteria range. Thus, these deviations were not considered to affect the interpretation of the results.
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	None
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: Acceptable

Executive summary
The objective of the present study was to assess the potential effects of glyphosate on the endocrine system, by identifying effects on pubertal development and thyroid function in the juvenile/peripubertal male rat. For this purpose, juvenile/peripubertal male Crl:CD(SD) Sprague Dawley rats (15 per dose group) were treated daily via oral gavage with glyphosate in 0.5 % methylcellulose in deionized water at doses of 0 (vehicle control), 100, 300, or 1000 mg/kg bw/day from postnatal day (PND) 23 to 53. Animals were observed twice daily for mortality and moribundity throughout the study period. Clinical observations and body weights were recorded daily. Animals were examined daily for preputial separation (PPS) beginning on PND 30. Age and weight at day of PPS were recorded. Following sacrifice on PND 53, blood was taken for total thyroxine (T4), testosterone, thyroid stimulating hormone (TSH), and clinical chemistry analysis. A gross necropsy was conducted for all animals and body and organ weights (epididymides, ventral prostate, dorsolateral prostate, adrenal glands, kidneys, liver, levator ani plus bulbocavernosus muscles, testes, pituitary, thyroid, seminal vesicle with coagulating gland) were recorded. Histopathological evaluation was performed on the thyroid, kidneys, testis and epididymides.

One male in the 1000 mg/kg bw/day dose group was found dead prior to dosing on PND 24. This death was considered treatment-related. All other males survived until scheduled sacrifice on PND 53. Rales were noted in a dose-related manner in the 300 and 1000 mg/kg bw/day dose groups approximately four hours post dosing throughout the treatment period. This finding persisted to the daily examinations for the 1000 mg/kg bw/day dose group. Terminal body weight was statistically significantly decreased for 300 and 1000 mg/kg bw/ day dose group animals compared to control. Body weight change (PND 23 to 53) was significantly decreased in the 300 and 1000 mg/kg bw/day dose group (-7.5 % and -12.4 %, respectively), reaching statistical significance in the highest dose group compared to control. Clinical signs of toxicity together with clearly reduced body weight/ body weight change are indicative of overt systemic toxicity.

A delay in the mean age of attainment of preputial separation (not statistically significant), decreased reproductive organ weights (epididymides, seminal vesicle with coagulating gland, and ventral prostate) (statistically significant), and a decreased mean testosterone level (not statistically significant) were noted in the 1000 mg/kg bw/day dose group. These observations were considered treatment-related but were considered to be a secondary non-specific consequence of the described systemic toxicity indicated by lower mean body weight in this dose group. No treatment-related effects on organ weights were observed at lower dosages. T4 and TSH levels were lower than those in the control group in all treated groups and testosterone was lower at 1000 mg/kg bw/day. However, these changes were not statistically significant and were not associated with any histopathological finding and were therefore not considered as compound-related. At 1000 mg/kg bw/day, the increases in ALT (also at 300 mg/kg bw/day), sodium, albumin, ALP, AST, chloride, phosphorous, and total protein, and the decrease in urea nitrogen were considered to be related to treatment and indicative for systemic toxicity. No adverse effects were noted on clinical pathology parameters at dosage levels of 100, 300, and 1000 mg/kg bw/day. No test substance-related macroscopic or microscopic findings were noted at any dose level.

There was no evidence of any glyphosate-related androgenic or anti-androgenic effects, nor was there any evidence of primary glyphosate-related effects on pubertal development or thyroid function in the juvenile/prepubertal male rat at dose levels of 100, 300, and 1000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White powder
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93% glyphosate acid 85.14% calculated glyphosate content
Stability of test compound:	Stable at room temperature

Vehicle:	Methylcellulose Source: Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA Lot/Batch No.: ZQ0344 CAS No.: 9004-67-5 Final concentration: 0.5 % in deionized water
Test animals:	
Species:	Rat
Strain:	Crl:CD(SD) Sprague Dawley
Source:	[redacted]
Age at start of dosing:	23 days
Sex:	Male
Weight at dosing:	44.3 - 56.6 g
Acclimation period:	Time-mated female Crl:CD(SD) rats (43 females) were received from [redacted] on 23 August 2011. Animals used in the present study were born at the testing facility. Weaning and study group assignment took place on PND 21.
Diet/Food:	Harlan Laboratories basal diet (2016*CM* Teklad Global, 16% Protein Rodent Diet; 11.0 ppm of total isoflavones (genistein + daidzein + glycitein), *ad libitum*
Water:	Reverse osmosis-purified drinking water, *ad libitum*
Housing:	Dams and littermates in plastic maternity cages with nesting material until weaning on PND 21. After weaning, juveniles were housed two males per cage throughout the study.
Environmental conditions:	Temperature: 21.2 - 22.2 °C Humidity: 40.1 - 58.9 % Air changes: 10/h Photocycle: 14 hours light/ 10 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 23 Aug 2011 delivery of pregnant rats; 29 Sep to 30 Oct 2011 test substance administration to male offspring

Animal assignment and treatment:
Offspring of time-mated female rats were weaned and weighed on PND 21. Animals were assigned using a WTDMS™ computer program which randomized the animals based on stratification of body weights in a block design. Littermates were not placed in the same group. The treatment groups included one vehicle control group and three glyphosate dose groups of 100, 300, and 1000 mg/kg bw/day.

All doses were administered once daily by oral gavage from PND 23 through PND 53, in a volume of 5.0 mL/kg bw. Dosing was performed daily, between 7:00 AM and 9:00 AM.

Table B.6.8.3.7-1: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted], 2012): Study design of glyphosate administration to juvenile male Sprague Dawley rats

Test group	Dose (mg/kg bw/day)[1]	No. of males	Dosing volume (mL/kg bw)
Control (vehicle)	0	15	5
Low	100	15	5
Mid	300	15	5
High	1000	15	5

[1] A correction factor of 85.14 % was used to account for the active substance.

Dose Preparation and Analysis
The vehicle (0.5% methylcellulose in deionized water) was prepared approximately weekly for administration to

the control group and for preparation of the test substance formulations; aliquots were prepared for daily dispensation to the control group and stored at room temperature.

The test substance formulations were prepared approximately weekly as single formulations for each dosage level, divided into aliquots for daily dispensation, and stored at room temperature.

Analyses to demonstrate homogeneity, resuspension homogeneity for suspensions, and stability of the test substance formulations for up to 15 days at room temperature storage at concentrations of 1 and 200 mg/mL were conducted prior to the start of dose administration in a separate study ([redacted]-843004). Dosing formulations were analysed five times during the study (September 27, 2011 and October 4, 11, 18 and 25, 2011). Analysed concentrations ranged from 97.5 - 102% of nominal for the low dose, 99.7 - 104% of nominal for the mid dose, and 104 - 114% of nominal for the high dose group. The analytical data indicated that the variation between nominal and actual dose to the animals was acceptable.

Mortality
All animals were examined twice daily, once in the morning and once in the afternoon, for mortality and moribundity.

Clinical observations
Clinical examinations were conducted on the day of randomization and daily through the day of necropsy. Animals were also observed for signs of toxicity in the morning prior to test substance administration and approximately 4 hours following test substance administration.

Body weight
Animals were weighed on the day of randomization, daily prior to test substance administration during the treatment period and on the day of necropsy.

Food consumption
Food consumption was not evaluated.

Preputial separation (PPS)
Beginning on PND 30, all animals were examined daily for onset of PPS. Age and body weight on the day at which complete PPS was observed was recorded for each animal. In addition, the appearance of a partial or complete PPS or a persistent thread of tissue between the glans and prepuce was recorded. If any animal within any group showed incomplete separation (persistent threads) for greater than three days, a separate second analysis was conducted using the age at which incomplete separation was first observed. Examination of the males was continued daily until complete PPS was attained or until euthanasia. When an animal did not attain PPS prior to necropsy, PND 54 (last study day + 1) and body weight at necropsy were used as the age and body weight at PPS, respectively.

Haematology and clinical chemistry
On PND 53 at least two hours post-dosing, rats were removed one at a time from their home cages to a separate room for euthanasia by decapitation without anaesthesia, to not induce stress-related responses in other animals, which may affect hormone measurements. All animals were euthanized by approximately 01:00 PM. Blood from the trunk of the animals was collected immediately into three serum separation tubes. One tube of serum samples was used for serum chemistry evaluation. The remaining two tubes of serum samples for each animal were used for T4, TSH, and testosterone analyses. Samples were analysed fresh or stored frozen (≤ -20 °C).

The following clinical chemistry parameters were examined: calcium, chloride, phosphorus, potassium, sodium, albumin, creatinine, urea nitrogen, total cholesterol, globulin (calculated), albumin/globulin ratio (calculated), glucose, total bilirubin, total protein, triglycerides, bile acids, alkaline phosphatase (ALP), alanine aminotransferase (ALT, also SGPT), aspartate aminotransferase (AST, also SGOT), gamma glutamyltransferase (GGT).

Testosterone and thyroxine (T4) concentrations were analysed using an electrochemiluminescent immunoassay. To determine the concentration of TSH in serum samples, a [^{125}I]rTSH kit was used.

Sacrifice and pathology
On PND 53 at least 2 hours post-dosing, rats were removed one at a time from their home cages to a separate

room for euthanasia by decapitation without anaesthesia. Gross necropsy was performed for all animals. The necropsy included examination of the external surface, all orifices, the external surface of the brain and the abdominal, thoracic, and pelvic cavities, including viscera. Any tissues with gross lesions were saved in 10% neutral-buffered formalin for possible future histopathologic examination. The following organs were collected and weighed: testes (left and right separately), epididymides (left and right separately), seminal vesicle with coagulating glands (with and without fluid), ventral prostate, dorsolateral prostate, levator ani plus bulbocavernosus (LABC) muscle complex, kidneys (paired), thyroid (paired, after fixation), liver, adrenal glands (paired), and pituitary gland. Subsequently, testes and epididymis were fixed in Bouin's solution overnight, then placed in 70 % ethanol until processing. The thyroid and kidneys were fixed in 10 % buffered formalin for at least 24 hours, then placed in 70 % ethanol until histopathological processing. Testes, epididymis, thyroid, and kidneys were routinely, histotechnically processed and haematoxylin and eosin stained slides were microscopically examined.

The thyroid sections (minimum of two sections) were subjectively evaluated for follicular epithelial height and colloid area and any abnormalities/ lesion were noted.

Performance criteria
Performance criteria as set out in the OCSPP test guideline 890.1500 were applied to evaluate measured values of the vehicle control group.

Statistics
Organ weights, organ weight to necropsy body weight ratio (liver, kidneys, pituitary, and adrenal glands), daily body weights (including necropsy body weight), cumulative body weight gain, serum hormones, serum chemistry parameters, and balanopreputial separation data each were tested for homogeneity of variance using Levene's test. For the purposes of this analysis, gamma glutamyltransferase values under range were assigned a value of 0.1 (half the lower limit of quantitation) for statistical analysis and reporting. Except for some daily body weight values and testosterone concentrations, all data were found to be homogeneous. For the non-homogenous data, a nonparametric test, as described below, was used to analyse the data.

When variances were homogeneous, an analysis of variance (ANOVA) was conducted on the raw data. The statistical model contained a factor for treatment group and a blocking factor based on the time of necropsy. A two-sided Dunnett's test was conducted using $p = 0.05$ to detect statistical significance, regardless of the outcome of the ANOVA, looking for significant differences in the test substance- treated groups when compared with the control group. For data that were not homogenous, the nonparametric Kruskal-Wallis test was used, ignoring the blocking factor, followed by Dunn's test, to compare each of the test substance-treated groups with the control group. The tests were two-sided at the 0.05 significance level, looking for significant differences from controls.

In addition, organ weights and balanopreputial separation data were subject to the following analyses: 1) Analysis of covariance (ANCOVA) with Dunnett's test. The model was as described above for ANOVA with the exception that PND 21 body weight was included as a covariate. 2) Linear trend test using the ANOVA model. 3) Linear trend test using the ANCOVA model.

Histopathology findings presented as a dichotomous response were analysed using pairwise Fisher's Exact tests to compare each treated group with the control. The tests were one-sided at the 0.05 significance level (testing for an increase). Histopathology findings presented as a graded response were analysed with pairwise Mann-Whitney U tests to compare each test substance-treated group with the control group. The tests were one-sided at the significance level testing for increased severity. The tests were two-sided for graded responses presented on a Grade 1-5 scale at the 0.05 significance level testing for increased or decreased severity. Exact p-values were calculated for the Mann-Whitney U test.

II. RESULTS AND DISCUSSION

A. MORTALITY
One animal of the 1000 mg/kg bw/day dose group was found dead in the morning on PND 24 (after one dose of glyphosate). Because clinical signs of toxicity and reduced body weight/ body weight change were dose-dependently observed in the 300 and 1000 mg/kg bw/day dose groups already following the first day of dosing, it cannot be excluded that this death was test substance-related. In addition, a death was observed in the 1000

mg/kg bw/day group in the 7-day pilot study. Thus, the unscheduled death observed at 1000 mg/kg bw/day was considered as test substance-related. All other males survived to scheduled necropsy.

B. CLINICAL OBSERVATIONS

A test substance-related clinical finding of rales was noted in a dose-related manner for males in the 300 and 1000 mg/kg bw/day dose groups approximately four hours post dosing (see table below). This condition persisted to the daily examinations primarily in the 1000 mg/kg bw/day dose group. This finding was noted on the first day of test substance administration, and continued sporadically throughout the treatment period until the day prior to necropsy. No other treatment-related clinical signs were noted in the dose groups compared to control.

Table B.6.8.3.7-2: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted] 2012): Incidence of clinical observations

Observation	Dose level (mg/kg bw/day)			
	Control	100	300	1000
Rales [1]	0 / 15	0 / 15	9 / 15	14 / 14

[1] Number animals with observation / number animals examined

C. BODY WEIGHT

Terminal body weight was statistically significantly decreased for 300 and 1000 mg/kg bw/day dose group animals (-6.63% and -10.36%, respectively) compared to terminal control group body weight (see table below). Body weight gain (PND 23 - 53) was significantly decreased in the 300 and 1000 mg/kg bw/day dose group (-7.5 % and -12.4 %, respectively), reaching statistical significance in the highest dose group, compared to control.

No test substance-related effects on mean body weights, daily body weight gains, or cumulative body weight gain were noted in the 100 mg/kg bw/day dose group.

The mean body weight value at the time of weaning (PND 21) in the control group (44.7 g) was slightly lower than the minimum value in the performance criteria (45.472 g); however, the coefficient of variation (CV) value was within the US EPA OPPTS 890.1500 guideline performance criteria range and the mean body weight value in the control group was similar to those in the test substance-treated groups on this day. Therefore, this out of range value was not considered to have affected interpretation of the body weight data.

Table B.6.8.3.7-3: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted], 2012): Body weight and cumulative body weight (bw) change in male Sprague Dawley rats exposed to glyphosate from PND 23 to PND 53 - selected timepoints

Body weight (g)								
Dose (mg/kg bw/day)	**Control**		**100**		**300**		**1000**	
PND	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
23	51.7	2.48	50.8	3.38	50.3	3.42	50.8	2.91
30	89.2	4.64	91.1	7.45	88.5	6.79	82.9*	8.36
37	141.6	7.21	145.5	13.56	139.0	9.88	134.2	12.34
44	201.3	11.23	203.1	21.80	191.1	15.67	189.0	15.96
51	259.8	12.99	261.0	25.08	238.8*	19.70	232.8*	23.35
53	273.1	14.02	276.3	25.37	255.0*	22.03	244.8*	23.18
BW change PND 23 - 53	-	-	-	-	-7.5 %	-	-12.4* %	-

PND = Postnatal day; SD = Standard deviation; * Significantly different from control at p <0.05; BW = Body weight

D. PREPUTIAL SEPARATION

On the day of attainment of complete PPS, mean body weights in all treatment groups were similar to the control group value. For the mean age of attainment of complete PPS, a not statistically significant delay was observed in the 1000 mg/kg bw/day dose group (48.0 days) when compared to the control group (45.9 days) (see table below). A statistically significant linear trend ($p \leq 0.0178$), which resulted from both the ANOVA (unadjusted value) and ANCOVA (adjusted value) models in the 1000 mg/kg/day dose group, was reported. When a second analysis was performed, adjusting the day of attainment for those males with three or more consecutive days of incomplete separation (persistent threads), no statistically significant delay in the mean age of attainment of preputial separation was noted for the 1000 mg/kg bw/day dose group when compared to the control group. There were 6, 11, 9, and 9 males in the control, 100, 300, and 1000 mg/kg bw/day dose group, respectively, with incomplete PSS for more than three days.

No test substance related effects were observed at doses ≤300 mg/kg bw/day.

The CV value for the body weight at the attainment of PPS in the control group (9.77%) was above the maximum value in the performance criteria (7.57%). However, the mean body weight value at the attainment of PPS and the mean and CV value for the age of attainment of PPS in the control group were within the acceptable US EPAOPPTS 890.1500 guideline performance criteria range.

It was previously demonstrated in the literature (Ashby and Lefevre, 2000[9]) that body weight differences of approximately 12% can result in a delay of PPS of one to two days. The delay observed in the 1000 mg/kg/day group occurred at a dose that produced a 10.36% lower final body weight. Thus, the observed reduced body weights of the 1000 mg/kg bw/day dose group may account for the delayed age at PPS, which would indicate rather a secondary than a primary treatment-related effect.

[9] Ashby J., & Lefevre, P.A. (2000). The peripubertal male rat assay as an alternative to the Hershberger castrated male rat assay for the detection of anti-androgens, oestrogens and metabolic modulators. *J. Appl. Toxicol*. 20:35-47.

Table B.6.8.3.7-4: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted], 2012): General growth and preputial separation (PPS)

Parameter evaluated		Vehicle Control				100 mg/kg bw/day				300 mg/kg bw/day				1000 mg/kg bw/day			
		n	Mean	±SD	CV	n	Mean	±SD	CV	n	Mean	±SD	CV	n	Mean	±SD	CV
Initial body weight (PND 23; g)	U	15	51.7	2.48	4.79	15	50.8	3.38	6.66	15	50.3	3.42	6.79	15	50.8	2.91	5.73
Body weight at PPS (g)	U	15	216.8	21.18	9.77	15	227.1	22.63	9.96	15	213.1	18.41	8.64	14	214.6	26.24	12.23
	A	15	216.5	21.18	9.77	15	226.9	22.63	9.96	15	213.1	18.41	8.64	14	214.9	26.24	12.23
Final body weight (g)	U	15	273.1	14.02	5.14	15	276.3	25.37	9.18	15	255.0*	22.03	8.64	14	244.8*	23.18	9.47
Final body weight (% of control)	U	-	-	-	-	-	1.17	-	-	-	-6.63	-	-	-	-10.36	-	-
Body weight gain (final – initial; g)	U	15	221.4	13.95	6.30	15	225.6	24.35	10.79	15	204.7	19.84	9.69	14	194.0*	21.59	11.13
	A	15	-	-	-	15	-	-	-	15	-	-	-	14	-	-	-
Age at PPS (PND)	U	15	45.9	2.17	4.72	15	46.9	2.42	5.16	15	47.4	3.07	6.47	14	48.0	1.66	3.47
	A	15	45.9	2.17	4.72	15	46.8	2.42	5.16	15	47.5	3.07	6.47	14	47.9[1]	1.66	3.47
Proportion unseparated (#/n)		0/15				0/15				1/15				0/14			

n = number of animals per group; SD = Standard deviation; CV = Coefficient of variation; PND = Postnatal day; U = Unadjusted for body weight on PND 23; A = Adjusted for body weight on PND 23; * Significantly different from control at $p < 0.05$, [1] Statistically significant based on a trend test.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

Blood clinical chemistry

Several serum chemistry parameters were statistically significantly changed (see table below). Higher mean alanine aminotransferase (ALT, 22.0% and 50.8%) and sodium (1.4% and 2.1%) levels were noted in the 300 and 1000 mg/kg bw/day groups, respectively, compared to control. Increased mean albumin (4.9%), alkaline phosphatase (ALP, 22.4%), aspartate aminotransferase (AST, 35.1%), chloride (4.0%), phosphorus (6.9%), and total protein (5.1%) levels were measured at 1000 mg/kg bw/day. Decreased (-16.9%) urea nitrogen level was noted in the 1000 mg/kg bw/day group compared to the control group. The changes were of a degree not to be considered toxicologically significant. In addition, the lower urea nitrogen was in a direction opposite to that normally associated with toxicological effects, and therefore was considered to represent normal biologic variability. The elevated albumin, sodium, and chloride may suggest dehydration, which is a nonspecific sign of systemic toxicity.

No other statistically significant differences from the control group were noted.

Hormone Levels

There were no test substance-related alterations in mean serum T4, TSH, and serum testosterone parameters compared to control (see table below). Mean serum T4 and TSH levels were decreased in all treatment groups compared to the control. However, none of the changes reached statistical significance and were not associated with any histological changes. TSH values were not dose-dependently changed.

The CV value for the mean TSH level in the control group (75.059%) exceeded the maximum value in the performance criteria (58.29%). However, the mean TSH value in the control group (8.30 ng/mL) was within the acceptable range in the US EPA OPPTS 890.1500 test guideline performance criteria (4.212 ng/mL to 24.112 ng/mL). Thus, this deviation was not considered to affect the final outcome of the study.

Table B.6.8.3.7-5: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted], 2012): Clinical chemistry – select parameters

	Vehicle (n=15)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=14)		
Parameter	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**
Albumin (g/dL)	4.1	0.12	3.05	4.1	0.14	3.27	4.1	0.18	4.35	4.3*	0.22	5.16
ALP (U/L)	277	36.8	13.3	276	34.5	12.5	287	59.6	20.8	339*	63.6	18.7
ALT (U/L)	59	7.7	13.0	66	8.7	13.2	72*	13.4	18.4	89*	23.1	26.0
AST (U/L)	111	17.1	15.4	129	21.7	16.8	131	28.6	21.9	150*	59.7	39.7
Chloride (mEq/L)	100	1.5	1.5	100	1.2	1.2	101	1.1	1.1	104*	2.4	2.3
Phosphorus (mg/dL)	10.1	0.61	6.03	10.3	0.61	5.90	10.4	0.70	6.77	10.8*	0.50	4.64
Sodium (mEq/L)	142	1.3	0.9	143	1.7	1.2	144*	1.3	0.9	145*	2.1	1.5
Total protein (g/dL)	5.9	0.21	3.57	6.0	0.21	3.47	6.0	0.21	3.49	6.2*	0.26	4.15
Urea nitrogen (mg/dL)	15.4	1.57	10.19	14.7	1.98	13.46	14.0	2.27	16.17	12.8*	2.36	18.43

SD = Standard deviation; CV = Coefficient of variation; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase;
* Significantly different from control at p <0.05

Table B.6.8.3.7-6: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted], 2012): Serum hormone concentrations

	Vehicle (n=15)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=14)		
Parameter	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**
Serum T4, total (µg/dL)	6.22	1.076	17.297	6.02	0.425	7.058	5.92	0.874	14.771	5.68	0.736	12.956
Serum TSH, (ng/mL)	8.30	6.230	75.059	6.77	3.728	55.039	6.91	2.816	40.771	5.37	3.396	63.224
Serum testosterone, total (ng/mL)	2.860	1.5697	54.8851	3.973	3.2270	81.2164	2.313	1.5352	66.3647	1.571	0.7966	50.6901

n = number animals per group; SD = Standard deviation; CV = Coefficient of variation; T4 = Thyroxine; TSH = Thyroid stimulating hormone

G. NECROPSY

Organ weights
Organ weights at necropsy are presented in the table below. Animals receiving 1000 mg/kg bw/ day glyphosate showed statistically reduced absolute liver (-15.1%), pituitary gland (-15.6%), LABC muscle (-15.9%), ventral prostate (-22.6%), and seminal vesicles with coagulating gland (-18.5%, with fluid) weights, when compared to control. Animals receiving 300 mg/kg bw/ day glyphosate showed statistically reduced absolute liver (-9.8%) and dorsolateral prostate (-14.3%) weights, compared to control. Organ weight changes were considered secondary to reduced body weights, rather than indicative for specific target organ toxicity of the test substance, because no histopathological correlates were identified. Other statistically significant differences from the control group were not observed in a dose-related manner, and therefore, were not considered test substance-related.

The mean thyroid gland (13.73 mg) and kidney (1.93 g) weights in the control group were below the minimum values (14 mg and 2.242 g, respectively) of the US EPA OPPTS 890.1500 test guideline performance criteria; however, the CV values were within the acceptable range. These deviations are not considered to affect the final outcome or validity of the present study.

Gross pathology
At scheduled necropsy, no test substance-related macroscopic findings were noted for treated animals, compared to control.

Histopathology
There were no test substance-related changes in the thyroid gland, epididymides, testes, or kidney. Specifically, histologic examination of testes and epididymides revealed no alteration in the tissue structure, degree of spermatogenesis (testes), or number of spermatids. With regard to thyroid gland, there was no difference in height of follicular epithelium or amount or nature of colloid within the individual follicles. All histologic changes were considered to be incidental findings.

Table B.6.8.3.7-7: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted], 2012): Organ weights – selected tissues

Organ		Control (n=15)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=14)		
		Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV
Adrenal glands (mg)	U	45.6	7.28	15.97	46.8	6.76	14.45	41.7	6.39	15.35	41.4	5.02	12.12
	A	45.4	7.28	15.97	46.9	6.76	14.45	41.5	6.39	15.35	41.7	5.02	12.12
Dorsolateral prostate (mg)	U	122.8	17.59	14.32	115.3	21.53	18.68	105.3*	15.41	14.64	112.2	18.87	16.81
	A	122.4	17.59	14.32	115.6	21.53	18.68	105.0*	15.41	14.64	113.0	18.87	16.81
Epididymis (left) (mg)	U	201.9	19.02	9.42	199.4	18.61	9.33	187.5	29.15	15.54	182.0	23.05	12.67
	A	201.2	19.02	9.42	199.3	18.61	9.33	187.2	29.15	15.54	183.0	23.05	12.67
Epididymis (right) (mg)	U	201.1	21.54	10.71	201.0	15.32	7.62	193.4	30.89	15.97	182.0	17.21	9.45
	A	200.2	21.54	10.71	201.2	15.32	7.62	192.9	30.89	15.97	183.4	17.21	9.45
LABC muscle group (mg)	U	539.6	61.50	11.40	527.8	107.65	20.40	491.4	97.15	19.77	453.9*	67.03	14.77
	A	537.9	61.50	11.40	528.9	107.65	20.40	490.0	97.15	19.77	457.2*	67.03	14.77
Liver (g)	U	12.69	1.064	8.384	13.09	1.563	11.937	11.45*	1.511	13.200	10.77*	1.506	13.985
	A	12.67	1.064	8.384	13.12	1.563	11.937	11.42*	1.511	13.200	10.82*	1.506	13.985
Pituitary (mg)	U	10.9	1.14	10.40	10.5	1.50	14.27	10.0	1.36	13.62	9.2*	1.71	18.59
	A	10.9	1.14	10.40	10.5	1.50	14.27	9.9	1.36	13.62	9.2*	1.71	18.59
Seminal vesicles w/ CG, w/o fluid (mg)	U	387.5	44.74	11.55	387.5	67.84	17.51	344.9	59.80	17.34	323.8*	52.43	16.19
	A	385.9	44.74	11.55	388.0	67.84	17.51	344.9	59.80	17.34	326.5*	52.43	16.19
Seminal vesicles w/ CG, w/ fluid (mg)	U	630.0	106.07	16.84	619.8	140.86	22.73	545.5	136.83	25.08	513.2*	88.22	17.19
	A	627.1	106.07	16.84	621.2	140.86	22.73	543.3	136.83	25.08	518.6*	88.22	17.19
Ventral prostate (mg)	U	257.5	37.75	14.66	264.0	61.11	23.14	236.3	44.33	18.76	199.2*	46.04	23.11
	A	256.5	37.75	14.66	264.8	61.11	23.14	235.4	44.33	18.76	201.2*	46.04	23.11

n = number of animals per group; SD = Standard deviation; CV = Coefficient of Variation; U = Unadjusted for body weight on postnatal day (PND) 23; A = Adjusted for body weight on PND 23; LABC = levator ani plus bulbocavernosus; * Significantly different from control at $p < 0.05$; w/ = with; w/o = without

III. CONCLUSIONS

Assessment and conclusion by applicant:
The study is performed in accordance with the current US EPA OPPTS/OCSPP 890.1500 (2009) where the following deviations were noted. Some values of the control group were outside the guideline performance criteria range (mean body weight at time of weaning (PND 21), CV value for body weight at attainment of PPS, CV value for mean TSH level, and mean thyroid gland and kidney weights). However, CV values of mean body weight (PND 21) and thyroid and kidney weights; mean body weight at the attainment of PPS and the mean/CV value for the age of attainment of PPS; and mean TSH value were in the acceptable guideline performance criteria range. Thus, these deviations were not considered to affect the interpretation of the results. The study is therefore considered valid. A delay in the mean age at attainment of balanopreputial separation, lower reproductive organ weights (epididymides, seminal vesicle with coagulating gland, and ventral prostate), and a lower mean testosterone level were noted in the 1000 mg/kg bw/day group. These changes at the limit dose were considered to be a secondary consequence of overt toxicity, noted by 10.36 % lower mean body weights and 12.4 % reduced mean body weight gain during the dosing period in this group versus the control group.
Thus, there was no evidence of any direct glyphosate-related androgenic or anti-androgenic effects, nor was there any evidence of direct glyphosate-related effects on pubertal development or thyroid function in the juvenile/ peripubertal male rat administered glyphosate up to the limit dose of 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

The conclusions made by the applicant are agreed with.

This conclusion is in line with the previous evaluation (addendum 2 to the RAR, 2017).
During the previous assessment is was stated that "*In this context it has to be noted, that the pubertal assays were not agreed upon at OECD level for methodological deficiencies, relativizing the validity of respective studies*" The RMS adds that even though the pubertal assays are not recommended in the testing strategy for endocrine activity of the EFSA/ECHA ED Guidance document, they are included in OECD 150 and mentioned in the guidance document as source of information that may be used or may be helpful for the assessment of ED properties. The EFSA/ECHA ED guidance document also mentions limitations noticed during their validation.

B.6.8.3.8. In vivo Pubertal assay in female rats

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats
Report No	[redacted]-843007
Document No	Not reported
Guidelines followed in study	US EPA OPPTS/OCSPP 890.1450 (2009)
Deviations from current test guideline (OCSPP 890.1450, 2009)	The mean age of vaginal opening attainment in the control group (PND 36.4) was higher than the acceptable range of values in the performance criteria (PND 30.67 to PND 35.62). However, the coefficient of variation (CV) value (6.52) fell within the acceptable range (0.00 - 6.52), and thus, met the acceptance criteria. The weights of adrenal glands of control animals (adjusted and unadjusted: 33.3 and 33.2, respectively) were slightly lower than the acceptable range (38.34 - 48.84 mg). These deviations were not considered to affect the validity or final outcome of the study.

Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	None
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: Study acceptable.

Executive summary
The objective of the study was to assess the potential effects of glyphosate on the endocrine system, by identifying effects on pubertal development and thyroid function in the juvenile/peripubertal female rat. For this purpose, 15 juvenile/peripubertal female Crl:CD(SD) Sprague Dawley rats per dose group were treated daily via oral gavage with glyphosate (in 0.5 % methylcellulose in deionized water) at doses of 0 (vehicle control), 100, 300, or the limit dose of 1000 mg/kg bw/day from postnatal day (PND) 22 to 42.

All females were observed twice daily for mortality and moribundity throughout the study. Clinical observations and body weights were recorded daily. All females were observed daily for vaginal opening. Once vaginal opening was observed, daily vaginal lavages were performed for each female to determine the stage of the estrous cycle. A complete necropsy was conducted on all rats euthanized in extremis or that survived to the scheduled euthanasia on PND 42. Adrenal glands, kidneys, liver, ovaries, pituitary gland, and thyroid were weighed and preserved at scheduled necropsy. Hormone (T4 and TSH) and clinical pathology evaluations (serum chemistry) were conducted on all surviving animals on PND 42. In addition, histopathological evaluation of the thyroid, kidney, ovary, and uterus was performed.

All treated females survived until terminal sacrifice. One female in the control group was found to have an impaired right forelimb on PND 27 and was sacrificed *in extremis*. Test substance-related rales were noted in a dose-related manner in the 300 and 1000 mg/kg bw/day dose groups. Rales were not noted in the control or the 100 mg/kg bw/day dose groups. Mean body weight and body weight change were unaffected by treatment. There were no treatment-related effects noted on the mean age of attainment of vaginal opening or body weight on the day of attainment. Mean estrous cycle lengths, was unaffected by treatment. There was a slight non-statistical increase in age at first estrus in the top dose. The percentage of regularly cycling females in the 300 and 1000 mg/kg bw/day groups was significantly ($p < 0.01$ or $p < 0.05$) lower than the control group. However, this was based on a low number of animals as a large number of animals had insufficient estrous cycle data. There were no treatment-related changes in serum chemistry, organ weights or serum hormone levels (T4 and TSH). No test substance-related macroscopic or microscopic findings were noted at any dosage level compared to control.

Although the effect on age at first estrus was not statistically significant and the effect on cycle regularity was not dose related and without dose response, the RMS considers that it cannot be fully excluded that these may be treatment related and therefore concludes the study to be equivocal in contrast to the study author who concluded the study to be negative.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White powder
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93% glyphosate acid 85.14% calculated glyphosate content
Stability of test compound:	Stable at room temperature

Vehicle:	Methylcellulose Source: Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA Lot/Batch No.: ZQ0344 CAS No.: 9004-67-5 Final concentration: 0.5% in deionized water
Test animals:	
Species:	Rat
Strain:	Crl:CD(SD) Sprague Dawley
Source:	[redacted]
Age at start of dosing:	22 days
Sex:	Female
Weight at dosing:	41.4 - 51.3 g
Acclimation period:	Time-mated female Crl:CD(SD) Sprague Dawley rats (43 females) were received from Charles River on 23 August 2011. The females were used to obtain the juvenile females for the present study. Hence, animals of the present study were born at the testing facility. Weaning took place on PND 21. Study allocation took place on PND 22.
Diet/Food:	Harlan Laboratories basal diet (2016*CM* Teklad Global, 16 % Protein Rodent Diet; 11.0 ppm of total isoflavones (genistein + daidzein + glycitein), *ad libitum*
Water:	Reverse-osmosis filtered tap water, *ad libitum*
Housing:	Dams and littermates in plastic maternity cages with nesting material until weaning on PND 21. After weaning, juveniles were housed two to three females per cage throughout the study.
Environmental conditions:	Temperature: 21.4 - 22.1 °C Humidity: 41.2 - 58.9% Air changes: 10/h Photocycle: 14 hours light/ 10 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 23 Aug 2011 delivery of pregnant rats; 28 Sep to 19 Oct 2011 test substance administration to female offspring

Animal assignment and treatment

Offspring of time-mated female rats were weaned and weighed on PND 21. Animals were assigned to study groups using a WTDMS™ computer program, which randomised the animals based on stratification of body weights in a block design. Littermates were not assigned to the same treatment group. The treatment groups included one vehicle control group and three glyphosate treatment groups at doses of 100, 300, and 1000 mg/kg bw/day.

All doses were administered once daily by oral gavage, from PND 22 through PND 42, in a volume of 5 mL/kg bw. Dosing was performed daily between 7:00 AM and 9:00 AM.

Table B.6.8.3.8-1: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted] 2012): Study design of pubertal development and thyroid function assay with glyphosate, administered orally to female Sprague Dawley rats

Test group	Dose (mg/kg bw/day)[1]	No. of females	Dosing volume (mL/kg bw/day)
Control (vehicle)	0	15	5
Low	100	15	5
Mid	300	15	5
High	1000	15	5

[1] A correction factor of 85.14 % was used to account for the active substance.

Dose Preparation and Analysis
The vehicle was prepared approximately weekly for administration to the control group and for preparation of the test substance formulations; aliquots were prepared for daily dispensation to the control group and stored at room temperature. The vehicle was mixed throughout the preparation, sampling, and dose administration procedures. The test substance formulations were prepared approximately weekly as single formulations for each dosage level by mixing appropriate amounts of test substance with 0.5 % methylcellulose in deionized water, divided into aliquots for daily dispensation, and stored at room temperature. The test substance formulations were continuously stirred throughout the preparation, sampling, and dose administration procedures.

Analyses to demonstrate homogeneity, resuspension homogeneity for suspensions, and stability of the test substance formulations for up to 15 days of room temperature storage at concentrations of 1 and 200 mg/mL were conducted prior to the start of dose administration in a separate study ([redacted]-843004). During the present study, samples of dose formulations were analysed three times (on September 27, October 4, and October 11, 2011). Analysed concentrations ranged from 98.9 - 100% of nominal for the low dose, 102 - 105% of nominal for the mid-dose, and 104 - 112% of nominal for the high dose. The analytical data indicated that the variation between nominal and actual dose to the animals was acceptable.

Mortality
All animals were examined twice daily, once in the morning and once in the afternoon, for mortality and moribundity.

Clinical observations
Clinical examinations were conducted on the day of randomization and daily (prior to test substance administration) through the day of scheduled necropsy. Animals were also observed for signs of toxicity approximately four hours following dose administration.

Body weight
Animals were weighed on the day of randomization, daily prior to test substance administration during the treatment period, and on the day of scheduled necropsy.

Food consumption
Food consumption was not evaluated.

Vaginal opening
Beginning on PND 22, all animals were examined daily for onset of vaginal opening. In addition, the appearance of a small “pin hole”, a vaginal thread, and complete vaginal opening were recorded. The age and body weight on the day at which the vaginal lumen was first observed to open was recorded for each animal. If any animal within any group showed incomplete opening (persistent pin holes and/or threads) for greater than three days, a separate second analysis was conducted using the age at which incomplete opening was first observed. Examination of the females was continued daily until vaginal opening was present or until euthanasia.

Estrous cyclicity
Beginning on the day of vaginal opening, up to and including the day of necropsy, daily vaginal lavages were performed. Slides were evaluated microscopically to determine the stage of the estrous cycle (proestrus [P], estrus [E], metestrus [M], diestrus [D]) of each female. The age at first vaginal estrus after vaginal opening was recorded. Females that had no E or P during the evaluation period were assigned an age of 43 days (last study day + 1 day). If a female had no E, but had a P during the evaluation period, the age at first estrus was considered the age on the day of P +1 day. The average cycle length was calculated and reported for complete estrous cycles (i.e. the total number of returns to M or D from E or P). Estrous cycle length was determined by counting the number of days from the first M or D in a cycle to the first M or D in a subsequent cycle.

At the end of the study, the overall pattern for each female was characterised as regularly cycling, irregularly cycling, not cycling, or insufficient data; based on predefined established definitions for these terms. For a “complete cycle”, an animal must have exhibited at minimum an E from the previous cycle, followed by one cycle (D to E), and a D from the start of the next cycle. The “percent cycling” and “percent cycling regularly” was calculated.

Haematology and clinical chemistry
On PND 42 approximately 2 h post-dosing (approximately 01:00 PM), rats were removed one at a time from their home cages to a separate room for euthanasia by decapitation without anaesthesia so as not to induce stress-related responses or diurnal fluctuations, which may affect hormone measurements. Blood from the trunk of the animals was collected immediately into three serum separation tubes. One tube of serum samples was used for serum chemistry evaluation. The remaining two tubes of serum samples for each animal were used for total thyroxine (T4) and thyroid stimulating hormone (TSH) analyses. Samples were analysed fresh or stored frozen (≤ -20 °C).
The following clinical chemistry parameters were examined: calcium, chloride, phosphorus, potassium, sodium, albumin, creatinine, urea nitrogen, total cholesterol, globulin (calculated), albumin/globulin ratio (calculated), glucose, total bilirubin, total protein, triglycerides, bile acids, alkaline phosphatase (ALP), alanine aminotransferase (ALT, also SGPT), aspartate aminotransferase (AST, also SGOT), gamma glutamyltransferase (GGT).

T4 and TSH levels were analysed using an electrochemiluminescent immunoassay and (^{125}I)rTSH kit, respectively.

Sacrifice and pathology
On PND 42 approximately 2 h post-dosing (at approximately 01:00 PM), rats were removed one at a time from their home cages to a separate room for euthanasia by decapitation without anaesthesia so as not to induce stress-related responses or diurnal fluctuations which may affect hormone measurements. The necropsy was conducted in a randomized manner and included examination of the external surface, all orifices, the external surface of the brain and the abdominal, thoracic, and pelvic cavities, including viscera. Any tissues with gross lesions were saved in 10% neutral-buffered formalin for possible future histopathologic examination. The following tissues were collected and weighed: ovaries (paired, without oviducts), uterus with cervix (both "wet" with luminal fluid and "blotted" following removal of luminal fluid), thyroid (weighed after fixation), adrenal glands (paired), pituitary, liver, and kidneys (paired). Additionally, the ovaries, uterus, thyroid and kidneys were fixed in 10% buffered formalin (ovaries and uterus stored in 70% ethanol prior to embedding) and subjected to histological processing and examination.

The thyroid sections (minimum of two sections) were subjectively evaluated for follicular epithelial height and colloid area and any abnormalities/lesions were noted. Five random sections from both ovaries were evaluated for follicular development and any abnormalities/lesions. The stage of the estrous cycle of a female at the time of necropsy was taken into account in the final histological assessment.

Statistics
Organ weights, organ weight to necropsy body weight ratio (liver, kidneys, pituitary, and adrenal glands), daily body weights (including necropsy body weight), cumulative body weight gain, serum chemistries, serum hormones and vaginal opening data were each was tested for homogeneity of variance using Levene's test. For the purposes of this analysis, gamma glutamyltransferase (GGT) values under range were assigned a value of 0.1 (half the lower limit of quantitation) for statistical analysis and reporting. Except for GGT concentrations, homogeneity of variance was confirmed for each endpoint ($p > 0.01$); AST concentrations required log transformation to demonstrate homogeneity.

An ANOVA was conducted on the raw or log-transformed homogenous data. The statistical model contained a factor for treatment group and a blocking factor based on the time of necropsy. A two-sided Dunnett's test was conducted, regardless of the outcome of the ANOVA, looking for significant differences in the test substance-treated groups when compared with the control group. For the GGT data, a nonparametric Kruskal-Wallis test was used, ignoring the blocking factor, followed by Dunn's test, to compare each of the test substance-treated groups with the control group. The tests were two-sided at the 0.05 significance level, looking for significant differences from controls.

In addition, organ weights and vaginal opening data were subject to the following analyses: 1) Analysis of covariance (ANCOVA) with Dunnett's test. The model was as described above for ANOVA with the exception that PND 21 body weight was included as a covariate; 2) Linear trend test using the ANOVA model; and 3) Linear trend test using the ANCOVA model.

Chi-square analysis was used to determine significant difference between cycling status (cycling vs. not cycling)

and percent of animals cycling regularly of the test substance-treated groups from the control group. Estrous cycle length and the day of first estrus were subjected to a parametric one-way ANOVA to determine intergroup differences. If the ANOVA revealed significant ($p < 0.05$) intergroup variance, Dunnett's test was used to compare the test substance-treated groups to the control group.

Histopathology findings presenting as a dichotomous response were analysed with pairwise Fisher's exact tests to compare each test substance-treated group with the control group. The tests were one-sided at the 0.05 significance level testing for an increase. Histopathology findings presented as a graded response were analysed with pairwise Mann-Whitney U tests to compare each treated group with the control. The tests were one-sided at the 0.05 significance level (testing for an increase). Tests were two-sided for graded responses presented on a grade 1-5 scale at 0.05 significance level testing for increased or decreased severity. Exact p-values were calculated for the Mann-Whitney U test.

II. RESULTS AND DISCUSSION

A. MORTALITY

All treated females survived until scheduled necropsy. Following dosing on PND 27, one control female was noted with impaired use of the right forelimb due to a possible mechanical injury. This female was subsequently euthanized *in extremis*.

B. CLINICAL OBSERVATIONS

Rales were noted for 4 and 13 females in the 300 and 1000 mg/kg bw/day dose group, respectively (see table below). They were observed approximately four hours following dose administration throughout the treatment period, and were considered treatment-related. Rales were neither observed in the control nor in the 100 mg/kg bw/day dose group. No other clinical signs were noted in the treatment groups compared to controls.

Table B.6.8.3.8-2: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted], 2012): Incidence of Clinical Observations

Observation	Dose Level (mg/kg bw/day)			
	Control	**100**	**300**	**1000**
Rales [1]	0 / 15	0 / 15	4 / 15	13 / 15

[1] Number animals with observation / number animals examined

C. BODY WEIGHT

No treatment-related effects on mean body weights or body weight changes were noted in the 100, 300, or 1000 mg/kg bw/day group when compared to the vehicle control group.

D. VAGINAL OPENING

Mean body weight and mean age at day of attainment of vaginal opening was not affected by treatment with 100, 300, or 1000 mg/kg bw/day glyphosate (see table below).

The mean age of vaginal opening attainment in the control group (PND 36.4) was higher than the acceptable range of values in the performance criteria (PND 30.67 to PND 35.62; US EPA OPPTS 890.1450). However, the coefficient of variation (CV) value (6.52) fell within the acceptable range (0.00 - 6.52), and thus, met the acceptance criteria. The increased mean age of vaginal opening of the control group was not considered to impact the validity or final outcome of the study.

Table B.6.8.3.8-3: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted], 2012): Body weight and vaginal opening (VO) - selected time points according to test guideline

Parameter Evaluated		Control				100 mg/kg bw/day				300 mg/kg bw/day				1000 mg/kg bw/day			
		n	**Mean**	**±SD**	**CV**	**n**	**Mean**	**±SD**	**CV**	**n**	**Mean**	**±SD**	**CV**	**n**	**Mean**	**±SD**	**CV**
Initial body weight (PND 22; g)	U	15	45.9	1.8	3.93	15	45.3	2.16	4.77	15	45.3	2.13	4.17	15	45.3	2.46	5.43
Body weight at vaginal opening (g)	U	14	119.6	12.62	10.56	15	119.2	16.25	13.64	15	125.1	15.57	12.44	15	117.4	16.80	14.31
	A	14	119.2	12.62	10.56	15	119.4	16.25	13.64	15	124.8	15.57	12.44	15	118.0	16.80	14.31
Final body weight (g)	U	14	147.3	11.68	7.93	15	147	10.3	7.01	15	152.1	6.37	4.19	15	141.3	10.60	7.50
Final body weight (% of control)	U	14	-	-	-	15	-0.20	-	-	15	3.25	-	-	15	-4.07	-	-
Body weight gain (g)	U	14	101.5	10.55	10.40	15	101.7	9.01	8.86	15	106.8	5.87	5.50	15	96.0	9.69	10.09
Age at vaginal opening (PND)	U	14	36.4	2.38	6.52	15	36.1	2.52	6.99	15	36.8	2.51	6.83	15	36.3	2.61	7.18
	A	14	36.5	2.38	6.52	15	36.1	2.52	6.99	15	36.8	2.51	6.83	15	36.3	2.61	7.18
Proportion unopened (animals affected/n)		**1/15**				**0/15**				**1/15**				**0/15**			

U = Unadjusted for body weight on PND 22; A = Adjusted for body weight on PND 22; n = Total number of animals per study group; SD = Standard Deviation; CV = Coefficient of variation; PND = Postnatal day

E. ESTROUS CYCLICITY

Mean estrous cycle lengths in the 100, 300, and 1000 mg/kg bw/day treated groups (4.8, 5.0, and 5.0 days, respectively) were similar to control group values (5.0 days) (see table below). No statistically significant differences in the age at the first occurrence of estrus were observed when the glyphosate-treated groups were compared to the control group although there does appear to be a slight non-significant increase at the top dose. The percentage of regularly cycling females in the 300 and 1000 mg/kg bw/day groups was significantly ($p < 0.01$ or $p < 0.05$) lower than the control group. However, a large number of females (up to ten per group) in the 300 and 1000 mg/kg bw/day groups had insufficient estrous cycle data (the animal did not display at least one complete estrous cycle but had at least one E and/or P and a partial cycle of five days or fewer, no E present on any days of estrous cycle determination and four or fewer days of data collected, or at least one E or P present and only one to four days of cycle data collection) that resulted in a limited number of females (five per group) available for evaluation of estrous cyclicity. These lower percentages did not occur in a dose-related manner and the percentage of females cycling in these groups was not statistically significantly different from the control group.

Table B.6.8.3.8-4: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted], 2012): Estrous cyclicity

Dose (mg/kg bw/day)	Number of animals	Mean age at first vaginal estrus (PND)	Mean cycle length (d)	Cycle status (regular/irregular/not-cycling/ insufficient data)	Cycling (%)	Regularly cycling (%)	Cycle status at sacrifice (# females)			
							Diestrus	Proestrus	Estrus	Not Cycling
Control	14	36.5	5.0	6/2/0/5	100	75	9	1	3	0
100	15	37.5	4.8	4/1/0/10	100	80	8	1	6	0
300	15	37.8	5.0	2/3/0/9	100	**40***	7	2	5	0
1000	15	38.3	5.0	3/2/0/10	100	**60***	11	0	4	0

PND = Postnatal day; * $p < 0.05$ as compared to control

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Blood clinical chemistry

There were no treatment-related changes in serum chemistry parameters that were considered adverse (see table below). The following statistically significant differences from control were observed: decreased AST levels in all three dose groups, decreased potassium levels in the 100 and 300 mg/kg bw/day dose groups, increased chloride and ALP levels in the 1000 mg/kg bw/day dose group, and decreased phosphorus levels in the 100 mg/kg bw/day dose group when compared to concurrent control. The changes of the ALP and chloride values in the 1000 mg/kg bw/day dose group were considered test substance-related because the elevations were limited to the high dose group. The changes in AST involved a decrease of normal levels, which is not usually associated with toxicological effects, and therefore the change was considered spurious and unrelated to the test substance. The decreased potassium and phosphorus levels occurred without a dose response-relationship and were considered to be of no biological relevance. Mean group potassium values, including the control group, were above the historical control data range of 3.97 - 5.94 mEq/L. However, this observation is considered to not influence the final outcome of the study.

Table B.6.8.3.8-5: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted], 2012): Selected clinical chemistry parameters

Parameter	Control (n=14)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=15)		
	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV
ALP (U/L)	252	30.8	12.2	260	51.1	19.7	283	38.1	13.5	303*	50.0	16.5
AST (U/L)	506	164.6	32.5	366*	89.7	24.5	374*	39.7	10.6	383*	109.6	28.6

Chloride (mEq/L)	100	1.6	1.6	101	1.0	1.0	100	1.7	1.7	103*	2.3	2.2
Phosphorus	11.7	1.00	8.56	10.8*	0.59	5.44	11.4	0.84	7.35	11.8	0.58	4.97
Potassium	8.08	0.977	12.08	7.00*	0.488	6.973	7.11*	0.558	7.850	7.55	0.638	8.455

SD = Standard deviation; CV = Coefficient of variation; ALP = alkaline phosphatase; AST = aspartate aminotransferase; * p

Hormone Levels
There were no treatment-related alterations in thyroid hormone parameters at any dose level tested (see table below). The T4 levels in the control group and the corresponding CV were within the US EPA OPPTS 890.1450 performance criteria.

Table B.6.8.3.8-6: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted], 2012): Hormone levels

Parameter	Control (n=14)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=15)		
	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV
Total T4 (µg/dL)	4.12	0.539	13.069	4.38	0.465	10.613	4.64	0.682	14.685	3.76	0.664	17.664
TSH (ng/mL)	3.74	1.917	51.207	3.86	1.627	42.115	3.85	2.172	56.452	2.69	1.265	47.095

SD = Standard deviation; CV = Coefficient of variation; T4 = Thyroxine; TSG = Thyroid stimulating hormone

G. NECROPSY

Organ weights
There were no test substance-related changes in weights of the ovaries (paired, without oviducts), uterus, kidneys (paired), thyroid, liver, adrenal glands (paired), and pituitary (see table below). The organ weights of the control animals met the performance criteria outlined in US EPA OPPTS 890.1450, except for adrenal weights (adjusted and unadjusted: 33.3 and 33.2, respectively), which were slightly lower than the acceptable range (38.34 - 48.84 mg). The CVs for organ weights were all within the performance criteria.

Gross pathology
No treatment-related macroscopic findings were observed.

Histopathology
There were no treatment-related microscopic findings in the ovaries, uterus, kidneys, or thyroid gland (see table below). All histologic changes were considered to be incidental findings and consistent with the age and strain of rats used.

Table B.6.8.3.8-7: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted], 2012): Organ weights

Organ weight (mg)		Control (n=14)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=15)		
		Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV
Adrenal glands, abs.	U	33.3	5.66	17.03	33.1	5.19	15.67	33.2	3.84	11.56	31.8	5.63	17.72
Adrenal glands, abs.	A	33.2	5.66	17.03	33.2	5.19	15.67	33.1	3.84	11.56	31.9	5.63	17.72
Adrenal glands, rel.	R	22.5	3.26	14.47	22.5	3.61	15.99	21.8	2.44	11.2	22.5	3.72	16.53
Kidneys, abs.	U	1170	104	8881	1160	119	10259	1170	100	8526	1140	102	8963
Kidneys, abs.	A	1160	104	8881	1160	119	10259	1170	100	8526	1150	102	8963
Kidneys, rel.	R	790	46	5853	790	49	6253	770	49	6340	810	57	7009
Liver, abs.	U	6720	851	12663	6750	649	9613	7030	469	6677	6470	524	8108
Liver, abs.	A	6700	851	12663	6760	649	9613	7010	469	6677	6500	524	8108
Liver, rel.	R	4550	321	7069	4580	219	4779	4620	204	4429	4580	184	4024
Ovaries, abs.	U	68.3	9.32	13.65	67.9	11.01	16.22	67.8	9.31	13.73	69.1	10.78	15.6
Ovaries, abs.	A	67.9	9.32	13.65	68.0	11.01	16.22	67.7	9.31	13.73	69.6	10.78	15.6
Pituitary, abs.	U	8.3	0.92	11.10	9.2	1.68	18.39	8.8	0.98	11.12	8.6	1.29	15.06
Pituitary, abs.	A	8.2	0.92	11.10	9.2	1.68	18.39	8.8	0.98	11.12	8.7	1.29	15.06
Pituitary, rel.	R	5.6	0.59	10.47	6.2	0.98	15.78	5.8	0.69	11.89	6.1	0.9	14.81
Thyroid glands, abs.	U	8.97	2.629	29.311	8.72	1.908	21.872	9.33	1.243	13.32	8.63	1.885	21.83
Thyroid glands, abs.	A	8.88	2.629	29.311	8.76	1.908	21.872	9.27	1.243	13.32	8.76	1.885	21.83
Uterus, blotted, abs.	U	235.7	68.25	28.95	256.8	56.78	22.11	260.2	72.29	27.78	205.9	73.09	35.5
Uterus, blotted, abs.	A	235.2	68.25	28.95	256.4	56.78	22.11	260.5	72.29	27.78	206.0	73.09	35.5
Uterus, wet, abs.	U	263.6	91.13	34.57	316.9	130.03	41.03	342.6	171.33	50.01	238.9	116.84	48.91
Uterus, wet, abs.	A	262.5	91.13	34.57	315.6	130.03	41.03	343.7	171.33	50.01	238.6	116.84	48.91

SD = Standard deviation; CV = Coefficient of variation; abs. = absolute; U = Unadjusted for body weight on PND 22; A = Adjusted for body weight on PND 22; rel. = relative; R = Organ-to-body weight ratio

Table 6.8.3-1: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted], 2012): Histopathological lesions – selected parameters

Findings		Control (n=14)		100 mg/kg bw/day (n=15)		300 mg/kg bw/day (n=15)		1000 mg/kg bw/day (n=15)	
		Observed	Examined	Observed	Examined	Observed	Examined	Observed	Examined
Ovaries									
Unremarkable		14	14	15	15	15	15	15	15
Uterus									
Unremarkable		14	14	15	15	15	15	15	15
Thyroid	Grade [1,]								
Colloid area	1	0	14	0	15	0	15	0	15
	2	0	14	0	15	0	15	0	15
	3	1	14	1	15	2	15	2	15
	4	10	14	8	15	8	15	9	15
	5	3	14	6	15	5	15	4	15
Follicular Cell Height	1	5	14	7	15	6	15	7	15
	2	9	14	8	15	9	15	8	15
	3	0	14	0	15	0	15	0	15
	4	0	14	0	15	0	15	0	15
	5	0	14	0	15	0	15	0	15

[1] Colloid area: 1 = most colloid, 5 = least colloid
[2] Follicular cell height: 1 = lowest, 5 = highest

III. CONCLUSIONS

Assessment and conclusion by applicant:
The study is performed in accordance with the current US EPA OPPTS/OCSPP 890.1450 (2009) where the following deviations were noted. The mean age of vaginal opening attainment in the control group was higher than the acceptable range of values in the performance criteria. However, the coefficient of variation value fell within the acceptable range, and thus, met the acceptance criteria. The weights of adrenal glands of control animals were slightly lower than the acceptable range. These deviations were not considered to impact the validity or final outcome of the study. The study is therefore considered valid. There was no evidence of any test substance-related estrogenic or anti-estrogenic effects, nor was there any evidence of test substance-related effects on pubertal development or thyroid function in the juvenile/peripubertal female rat following oral administration of glyphosate at dosage levels of 100, 300, and 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

Most of the parameters investigated did not give an indication of an endocrine effect. However, a decrease in regularity of estrous cycle was observed at 300 and 1000 mg/kg bw/day. The effect was based on a small number of animals and did not show a clear dose-response. In addition, a slight non-significant increase in the age at first estrus was observed. Overall, the study is concluded to be equivocal by the RMS.

This conclusion is in line with the previous evaluation (addendum 2 to the RAR, 2017).
During the previous assessment is was stated that "*In this context it has to be noted, that the pubertal assays were not agreed upon at OECD level for methodological deficiencies, relativizing the validity of respective studies*" The RMS adds that even though the pubertal assays are not recommended in the testing strategy for endocrine activity of the EFSA/ECHA ED Guidance document, they are included in OECD 150 and mentioned in the guidance document as source of information that may be used or may be helpful for the assessment of ED properties. The EFSA/ECHA ED guidance document also mentions limitations noticed during their validation.

B.6.8.3.9. Public literature - In vitro steroidogenesis

Data point:	CA 5.8.3
Report author	Hecker, M. *et al.*
Report year	2011
Report title	The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter-laboratory validation study
Report No	DOI 10.1007/s11356-010-0396-x
Document No	Not reported
Guidelines followed in study	Not applicable (OECD Test Guideline (TG) was approved in July 2011 as OECD TG 456, after the work of the publication was completed).
Deviations from current test guideline (OCSPP 890.1550, 2009) (OECD 456, 2011)	None (key values provided in the publication are in line with current OECD TG 456).
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Not specified
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: Study acceptable

2. Full summary

Introduction: The purpose of this study was to validate a standardised steroidogenesis assay protocol to develop an OECD draft test guideline. (Note: The OECD Test Guideline (TG) was approved in July 2011 as OECD TG 456.) Twenty-eight chemicals were selected as model substances to validate the H295R steroidogenesis assay, to identify potential effects of endocrine-disrupting substances on the production of testosterone (T) and 17β-estradiol (E2). These test substances were selected based on their known or suspected endocrine activity, or lack thereof, and included inhibitors and inducers of different potencies as well as positive and negative controls. They were selected and approved by the OECD Validation and Management Group for Non-Animal Testing (VMG NA). Glyphosate was one of the test substances evaluated. A total of seven laboratories from the US, Denmark, Germany, Japan, Hong Kong, and Canada participated in this validation study. Two of these participating laboratories evaluated glyphosate.

Materials and methods: H295R cells were cultured under standard cell culture conditions for at least four or five passages prior to use in the assay. The cells were plated into 24-well cell culture plates at a density of approximately 200,000 to 300,000 cells/mL. Cells were allowed to acclimate for 24 hours prior to test substance exposure. Then, cells were exposed for 48 hours to seven concentrations of glyphosate using log intervals between 0.0001 and 100 μM of the test substance in triplicate. A concurrent quality control plate was included in each of the independent runs to demonstrate the correct assay's response to forskolin (a known inducer of testosterone and estradiol production) and prochloraz (a known inhibitor of testosterone and estradiol production). After the 48-hour exposure period, medium was collected from all wells and concentrations of hormones were measured using commercially available hormone detection kits (in one laboratory using enzyme linked immunoassay and liquid chromatography with mass spectroscopy [hormones were extracted with ethyl ether before analysis], in the other laboratory using radio immunoassay without previous extraction of the hormones). After media removal, cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay or the Live/Dead® variability assay. All concentrations with cell viability ≤80%, were excluded from the data analysis. One laboratory conducted one experiment without further replicates; the other laboratory conducted three independent experiments with the test substance glyphosate. All laboratories were required to demonstrate proficiency in performing the single procedures that are part of the H295R steroidogenesis assay protocol. For this purpose, predefined performance criteria had to be met. In addition, each test substance was tested for potential interference with the hormone detection system used before exposing cells in the main experiment. This was of particular relevance for antibody-based assays such as the enzyme linked immunoassay and the radioimmunoassay because it has been previously shown that some chemicals can interfere with these tests.

To examine the relative changes in hormone production, results were normalized to the mean vehicle control (VC) value, and results were expressed as percent change relative to the VC. Prior to conducting statistical analyses, the assumptions of data normality and variance of homogeneity were evaluated. Normality was evaluated using standard probability plots or the Shapiro–Wilk's test. If the data were normally distributed, differences between chemical treatments and vehicle controls were analysed using one-way analysis of variance (ANOVA) followed by a two-sided Dunnett's test. If data were not normally distributed, the Kruskal–Wallis test followed by the Mann–Whitney U test were used. Data analysis was conducted using pooled replicate experiments. Differences were considered significant at $p < 0.05$.

Results: In the present H295R steroidogenesis assay validation study, glyphosate was tested negative for changes in T, as well as E2 production. None of the two laboratories could detect a change in hormone production when compared to the concurrent control. The authors stated that test substances that tested negative for T and E2 effects *in vitro* (H295R assay), like glyphosate, did not cause any changes in serum T and E2 concentrations *in vivo*, too (Soso *et al.*, 2007[10]). This result is consistent with an article in the peer-reviewed literature using similar methodology in Leydig cells (Forgacs *et al.*, 2012[11]).

[10] Soso, A.B, Barcellos, L.J.G., Ranzani-Paiva, M.J. *et al.* (2007). Chronic exposure to sub-lethal concentrations of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundi'a (*Rhamdia quelen*). *Environ Toxicol Pharm*. 23:308-313.

[11] Forgacs A.L., Ding, Q., Jaremba, R.G., *et al.* (2012). BLTK1 murine Leydig cells: A novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. *Toxicol. Sci*. 127:391-402.

In conclusion, glyphosate was tested negative for changes in E2 and T production in the H295R steroidogenesis assay in two separate laboratories during OECD's Phase 3 validation.

CONCLUSIONS

Assessment and conclusion by applicant:
Key values provided in the publication are in line with current OECD 456 (2011). The study is therefore considered valid. This OECD inter-laboratory validation of the H295R steroidogenesis assay, which includes glyphosate as one of many test materials, demonstrates that glyphosate does not impact 17β-estradiol or testosterone production.

Assessment and conclusion by RMS:
The conclusion made by the applicant is agreed with. This conclusion is in line with the previous EU evaluation (addendum 2 to the RAR, 2017).

B.6.8.3.10. Public literature – QSAR screening for ED

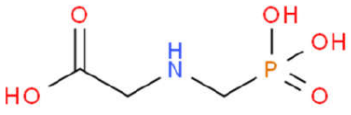
Data point	CA 5.8.3
Report author	[redacted]
Report year	2020
Report title	(Q)SAR screening on endocrine disrupting potential of Glyphosate under the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009
Report No	110517-1
Document No	Not reported
Guidelines followed in study	Not applicable. Assessment performed according to the guidance document *Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009* (ECHA-EFSA, 2018).
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability	Conclusion GRG: Valid, Category 1 Conclusion AGG: Study acceptable.

(Q)SAR predictions were generated using selected publicly available and commercial models. Five QSAR tools were applied for predictions of potential endocrine activity of glyphosate: OECD QSAR Toolbox, Vega, Endocrine Disruptome, Danish QSAR database and ToxCast COMPARA/CERAPP consensus models. The list of investigated receptors include: estrogen receptor (ER), androgen receptor (AR), thyroid receptor (TR), glycocorticoid receptor (GR), mineralocorticoid receptor (MR), liver X receptor (LXR), peroxisome proliferator-activated receptor (PPAR), retinoid X receptor (RXR), aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), and CYP3A4 receptor.

Materials and methods

Test compound: The evaluated compound is glyphosate (N-(phosphonomethyl)glycine).

Table 1: Basic information on the evaluated substance.

Substance name (CAS No.)	Chemical structure	Molecular formula	Smiles code
Glyphosate (CAS 1071-83-6)		$C_3H_8N_1O_5P_1$	C(=O)(O)CNCP(=O)(O)O

Models:
Five freely available software packages/models for QSAR evaluation were selected: OECD QSAR Toolbox v4.3, Vega v1.1.5, Danish QSAR database, Endocrine Disruptome and OPERA Consesus models COMPARA/CERAPP.

Details on the models are provided in the study report.

Results

1. Potential estrogenic activity

Table B.6.8.3.10-1: (Q)SAR screening on endocrine disrupting potential of Glyphosate: Outcome of the overall *in silico* screening for estrogen activity

Model	Outcome	Applicability domain compliance	Remarks	Reliability of prediction
OECD Toolbox ER binding	Non-binder, non cyclic structure	n.a.	No indication for estrogenic activity	Medium
OECD Toolbox rtER Expert System	No alert found	n.a.	No indication for estrogenic activity	Medium
OECD Toolbox DART	Not known precedent reproductive and developmental toxicant	n.a.	No indication for estrogenic activity	Medium
Vega ER relative binding affinity	Inactive	Out	No indication for estrogenic activity	Low
Vega Estrogen Receptormediated effect (IRFMN/CERAPP)	Possible non-active	Out	One alert for possible non-activity was found.	Low
Endocrine Disruptome ER alpha	-4.3 (low probability of binding	n.a.	No indication for estrogenic activity	Medium
Endocrine Disruptome ER alpha an.	-3.8 (low probability of binding	n.a.	No indication for estrogenic activity	Medium
Endocrine Disruptome ER beta	-4.6 (low probability of binding	n.a.	No indication for estrogenic activity	Medium
Endocrine Disruptome ER beta an.	-3.8 (low probability of binding	n.a.	No indication for estrogenic activity	Medium
Danish QSAR DB ER alpha binding complete training set, battery	Negative	In	No indication for estrogenic activity	High
Danish QSAR DB ER	Inconclusive	Out	No indication for	Low

alpha binding complete training set, MultiCase			estrogenic activity	
Danish QSAR DB ER alpha binding complete training set, Leadscope	Negative	In	No indication for estrogenic activity	High
Danish QSAR DB ER alpha binding complete training set, SciQSAR	Negative	In	No indication for estrogenic activity	High
Danish QSAR DB ER alpha binding balanced training set, battery	Negative	In	No indication for estrogenic activity	High
Danish QSAR DB ER alpha binding balanced training set, MultiCase	Inconclusive	Out	No indication for estrogenic activity	Low
Danish QSAR DB ER alpha binding balanced training set, Leadscope	Negative	In	No indication for estrogenic activity	High
Danish QSAR DB ER alpha binding balanced training set, SciQSAR	Negative	In	No indication for estrogenic activity	High
Danish QSAR DB ER alpha activation, battery	Inconclusive	Out	No indication for estrogenic activity	Low
Danish QSAR DB ER alpha activation, MultiCase	Inconclusive	Out	No indication for estrogenic activity	Low
Danish QSAR DB ER alpha activation, Leadscope	Negative	Out	No indication for estrogenic activity	Low
Danish QSAR DB ER alpha activation, SciQSAR	Negative	Out	No indication for estrogenic activity	Low
CERAPP Consensus ER / Agonist	Inactive	In	CERAPP consensus predictions have the highest reliability among all predictions as presented in this table.	High
CERAPP Consensus ER / Antagonist	Inactive	In	CERAPP consensus predictions have the highest reliability among all predictions as presented in this table.	High
CERAPP Consensus ER / Binding	Inactive	In	No indication for estrogenic activity (Glyphosate is included in the training set with	High

			negative results).	

2. Potential Androgenic activity

Table B.6.8.3.10-2: (Q)SAR screening on endocrine disrupting potential of Glyphosate: Outcome of the overall *in silico* screening for androgen activity

Model	Outcome	Applicability domain compliance	Remarks	Reliability of prediction
OECD Toolbox DART	Not known precedent reproductive and developmental toxicant	n.a.	No indication for androgenic activity	Medium
Vega Androgen Receptormediated effect (IRFMN/CoMPARA)	Inactive	Out	No indication for androgenic activity	Low
Endocrine Disruptome AR	-4.9 (low probability of binding	n.a.	No indication for androgenic activity	Medium
Endocrine Disruptome AR an.	-4.4 (medium probability of binding	n.a.	Low indication for androgenic activity	Medium
Danish QSAR DB AR antagonism, battery	Negative	In	No indication for androgenic activity (Glyphosate is included in the training set with negative results)	High
Danish QSAR DB AR antagonism, MultiCase	Negative	In	No indication for androgenic activity (Glyphosate is included in the training set with negative results)	High
Danish QSAR DB AR antagonism, Leadscope	Negative	In	No indication for androgenic activity (Glyphosate is included in the training set with negative results)	High
Danish QSAR DB AR antagonism, SciQSAR	Negative	In	No indication for androgenic activity (Glyphosate is included in the training set with negative results)	High
COMPARA (Consensus) Agonist	Inactive	In	CoMPARA consensus predictions have the highest reliability among all predictions as presented in this table.	High
COMPARA (Consensus) Antagonist	Inactive	In	CoMPARA consensus predictions have the highest reliability among all predictions as presented in this table.	High
COMPARA (Consensus) Binding	Inactive	In	CoMPARA consensus predictions have the highest reliability among all predictions as presented in this	High

			table.	

3. Potential Steroid activity

Table B.6.8.3.10-3: (Q)SAR screening on endocrine disrupting potential of Glyphosate: Outcome of the overall *in silico* screening for steroid activity

Model	Outcome	Applicability domain compliance	Remarks	Reliability of prediction
Endocrine Disruptome glucocorticoid receptor (GR)	-4.8 (low probability of binding)	n.a.	No indication for steroid activity	Medium
Endocrine Disruptome GR antagonism	-3.9 (low probability of binding)	n.a.	No indication for steroid activity	Medium
Endocrine Disruptome Mineralocorticoid receptor (MR)	-4.4 (low probability of binding)	n.a.	No indication for steroid activity	Medium

4. Potential thyroid activity

Model	Outcome	Applicability domain compliance	Remarks	Reliability of prediction
Endocrine Disruptome TR alpha	-4.5 (low probability of binding)	n.a.	No indication for steroid activity	Medium
Endocrine Disruptome TR beta	-4.4 (low probability of binding)	n.a.	No indication for steroid activity	Medium
Danish QSAR DB TPO inhibition QSAR1, Leadscope	Inconclusive	Out	No indication for steroid activity	Low
Danish QSAR DB TPO inhibition QSAR2, Leadscope	Negative	Out	No indication for steroid activity	Low

5. Overall assessment:

Table B.6.8.3.10-1: (Q)SAR screening on endocrine disrupting potential of Glyphosate: Outcome of the overall *in silico* screening for glyphosate

Modality	Summary outcome of *in silico* screening	Remarks
Estrogen	No indication	Estrogenic activity was predicted negative with all five applied models. Due to the high amount of data available on ER activity, the high quality of CERAPP Consensus predictions and glyphosate being part of the training set for ER binding tests, the prediction of ER activity of glyphosate was considered reliable.
Androgen	No indication	Androgenic activity was predicted negative with all five applied models. Due to the quality of CoMPARA consensus predictions in combination with other models (predicting no androgenic activity), the assessment of androgenic activity based on the available models was considered reliable. This is further strengthened, as glyphosate is part of the testing battery of the Danish QSAR DB and tested negative for antagonistic effect on the

		human androgen receptor *in vitro*.
Steroid	No indication	There are three results available for steroid receptors: glucocorticoid receptor (GR) and glucocorticoid receptor antagonism and mineralocorticoid receptor (MR). No steroid activity is predicted for all three receptors by the molecular docking method (Endocrine Disruptome).
Thyroid	No indication	TR binding activity is predicted to be low for glyphosate by the molecular docking method (Endocrine Disruptome). Two models available in the Danish QSAR database yield inconclusive and negative results. Both predictions are out of applicability domain and thus of low reliability.
Other	No indication	Overall, there is no indication of activity for endocrine activities other than estrogen, androgen, steroid and thyroid, however due to the general lack of models for the various receptors, the result should be considered with caution.

CONCLUSIONS

Assessment and conclusion by applicant:
Five QSAR tools were applied for predictions of potential endocrine activity of glyphosate: OECD QSAR Toolbox, Vega, Endocrine Disruptome, Danish QSAR database and OPERA ToxCast COMPARA/CERAPP consensus models.
For androgenic and estrogenic activity, the largest number of data and models is available. Specifically due to the available CONSENSUS models (CoMPARA/CERAPP) derived from the extensive high-throughput screening tests as conducted under the EDSP in combination with available literature derived predictions, the overall assessment of androgenic and estrogenic activities is considered reliable and was shown to be negative. This outcome is strengthened because glyphosate is part of the training set of ER binding tests showing negative results as well as tested negative for antagonistic effects on the human androgen receptor *in vitro*.
For all other modalities, lower amount of data and models are available. Thus, the confidence in the screening assessments for thyroid, steroid or other modalities is lower. However, also here no indication for thyroid, steroid or other modalities activities were observed.
Overall, there is no indication that glyphosate has endocrine disrupting activity.
For details please refer to the (Q)SAR ED assessment report *(Q)SAR screening on endocrine disrupting potential of Glyphosate under the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009*.

Assessment and conclusion by RMS:

The results of the QSAR analysis do not indicate a potential ED concern.

B.6.8.3.11. Public literature – Effect of glyphosate of granulosa cells of swine ovaries and adipose stromal cells

Data point:	CA 5.8.3
Report author	Gigante, P. *et al.*
Report year	2018
Report title	Glyphosate affects swine ovarian and adipose stromal cell functions
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Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable (Klimisch Score 1)

Executive Summary
The effect of glyphosate (GLY) at different doses (0.2, 4 and 16 µg/mL) was evaluated on granulosa cells growth (BrDU incorporation and ATP production), steroidogenesis (17-β estradiol and progesterone secretion) and redox status (superoxide and nitric oxide production and non-enzymatic scavenging activity). GLY has been shown to inhibit cell growth, 17-β estradiol and non-enzymatic scavenging activity and to increase progesterone and nitric oxide secretion ($P < 0.05$). In addition, GLY significantly decreased the viability of ASCs ($P < 0.001$), and inhibited their adipogenic differentiation. The authors concluded that these data indicate that GLY alters the main features of granulosa cells and ASCs; thus suggesting that GLY could affect both reproductive function and adipose tissues homeostasis.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material
Glyphosate was purchased from Sigma Chemical Co, St Louis, USA. The purity was not reported.

Isolation and culture of granulosa cells
Swine ovaries were collected at a local slaughterhouse, placed in PBS at 4 °C supplemented with penicillin (100 Ul/mL), streptomycin (100 Ul/mL) and amphotericin B (2.5 µg/mL) and transported to the laboratory within 1 hour. The samples were immersed for 1 minute in ethanol 70 % before processing. Cystic or hemorrhagic follicles were discarded and the granulosa cells were harvested by aspiration of the follicles in the later state of maturation. The granulosa cells were then centrifuged at 450xg for 10 minutes and the cell pellet obtained treated with ammonium chloride to remove red blood cells. The cell number was estimated after vital staining with trypan blue whereafter the cells were plated and cultured in a validated serum free system composed of DMEM/Ham's F12 medium supplemented with penicillin (100 µg/mL), amphotericin B (2,5 µg/mL), streptomycin (100 µg/mL), sodium selenite (5 ng/mL) and transferrin (5 µg/mL) (CM). CM medium maintains the characteristics of granulosa cells and avoids luteinization.

B. STUDY DESIGN

Cell proliferation
Cell proliferation is measured using the BrdU cell proliferation ELISA assay. The granulosa cells were plated into 96-well plates (10^4 cells/100 µL CM) and incubated overnight with glyphosate at 0, 0.2, 4 or16 µg/mL. At the end, plates were centrifuged for 10 minutes at 400×g, the supernatants discarded and the cells dried and fixed. The DNA was denatured before the addition of the anti-BrdU antibody, conjugated with horseradish peroxidase (POD). The POD substrate used was tetramethyl-benzidine (TMB). Absorbance at 450 nm of the blue color formed is proportional to the amount of newly synthesized DNA. To quantify the viable cell number, the absorbance of each sample was related to a standard curve prepared by culturing, in quintuplicate, granulosa cells at different plating densities for 48 hours. The curve was repeated in four different experiments. The cell number/well was estimated from the resulting linear regression equation and was used to correct experimental data. The assay detection limit was 10^3 cell/well and the variation coeffcient was less than 5 %.

Cell viability
Granulosa cell viability was assessed using the ATP-lite bioluminescent assay based on the reaction of ATP luciferase and luciferin. The light emitted is proportional to the ATP concentration in the cells. The test was validated by plating different viable cell numbers (from 2.5×10^3 to 4×10^6/100 µL) three times. The relationship between cell number and luminescence was linear ($r = 0.95$). 2×10^5 cells/100 µL CM were seeded in 96-well plates and treated with glyphosate at 0, 0.2, 4 or 16 µg/mL for 48 hours. Kit reagents were added according to the instructions and luminescence was measured.

Granulosa cell steroid production
Granulosa cells at 10^4/200 µL in CM supplemented with androstenedione at 28 ng/mL were seeded in 96-well

plates and treated with glyphosate at 0.2, 4 or16 µg/mL. After 48 hours incubation, culture media were collected, frozen and stored at −20 °C until determination of progesterone (P4) and 17-β estradiol (E2) by a validated RIA. P4 assay sensitivity and ED_{50} were 0.24 and 1 nmol/L, respectively. E2 assay sensitivity and ED_{50} were 0.05 and 0.2 nmol/L. The intra- and inter-assay coefficients of variation were less than 12 % for both assays.

Granulosa cell superoxide (O_2^-) production

O_2^--production was measured using the WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolium]-1,3-benzene disulfonate) test which is based on the cleavage of the water soluble tetrazolium salt, WST-1, to the water soluble formazan. A total of 10^4 cells/200 µL CM were seeded in 96-well plates and incubated with glyphosate at 0, 0.2, 4 or 16 µg/mL for 48 hours. During the last 4 hours of incubation, 20 µL of WST-1 was added to the cells, and the absorbance determined at 450 nm against 620 nm. The coefficients of variation were less than 3%.

Granulosa cell nitric oxide (NO) production

10^5 viable cells/200 µL CM were seeded in 96-well plates and treated with glyphosate at 0.2, 4 or 16 µg/mL for 48 hours. At the end, plates were centrifuged for 10 minutes at 400×g, the supernatants were collected and NO levels assessed by measuring nitrite after incubation with Greiss reagent (1 % sulfanilamide, 5 % phosphoric acid and 0.1 % N-[naphtyl] ethylenediaminedihydrochloride). The absorbance was measured at 540 nm against 620 nm. A calibration curve ranging from 25 to 0.39 µM was prepared by diluting sodium nitrite in CM.

Non-enzymatic scavenging activity

The Ferric Reducing Activity of Plasma (FRAP) assay is a colorimetric method based on the ability of the antioxidant molecules present in a biological matrix to reduce ferric-tripiridyltriazine (Fe^{3+} TPTZ) to a ferrous form (Fe^{2+} TPTZ). 2×10^5 cells/200 µL CM were seeded in 96-well plates and treated with glyphosate at 0, 0.2, 4 or 16 µg/mL. At the end, the plates were centrifuged for 10 minutes at 400×g, the supernatants discarded and the cells lysed by adding, Triton 0.5 % + PMSF in PBS at 200 µL/well in an ice bath for 30 minutes. The test was performed on 40 µL of cell lysates added to Fe^{3+} TPTZ reagent. After 30 minutes incubation at 37 °C, absorbance of Fe^{2+} TPTZ was determined at 595 nm. The ferric reducing ability of cell lysates was calculated by plotting a standard curve of absorbance against $FeSO_4$ - $7H_2O$ standard solution.

Isolation and culture of adipose stromal cells (ASCs) from swine adipose tissue

Samples of about 5 g of subcutaneous abdominal adipose tissue were collected from pigs at a local slaughterhouse and placed in 20 mL of PBS supplemented with penicillin (100 Ul/mL), streptomycin (100 Ul / mL) and amphotericin B (2.5 µg/mL), transferred to Petri dishes in sterile conditions and shredded in fragments of about 3 mm^3. Thereafter, the adipose tissue fragments were distributed in 6-well plates, at a density of 10 fragments/well and then carefully covered with a minimal quantity (1 mL) of culture medium (CM_{ASC}) to avoid floating. CM_{ASC} was composed of low glucose DMEM + GlutaMax, supplemented with 10% FBS, penicillin (100 UI/mL), streptomycin (100 UI/mL) and amphotericin B (2.5 µg/mL). The plates were maintained at 37 °C in a humidified atmosphere at 5% CO_2 and 19% O_2. Every 48 hours CM_{ASC} was replaced. After 5 to 7 days, the adipose tissue fragments were removed from the culture plates and the remaining adherent cells, growing in monolayer, were cultured in fresh CM_{ASC} for 5 to 7 days, until 80 % confluence was reached. The ASCs were the trypsinized and cultured in 25 cm^2 flasks with 5 mL CM_{ASC}. The ASCs obtained were subsequently either used to evaluate the expression of adipogenenesis-related marker genes or plated to study the effects of glyphosate at 4 µg/mL on cell viability and adipogenic differentiation.

Adipose stromal cells (ASCs) viability

Cell viability was evaluated using the MTT assay. ASCs were seeded in 96-well plates at 10^4 cells/well in 200 µL CM_{ASC} and incubated at 37 °C in humidified atmosphere at 5 % CO_2 and 19 % O_2 for 48 and 72 hours with glyphosate at 0 or 4 µg/mL. At the end of each incubation period, 20 µL of 5 mg/mL of MTT were added and incubated for 4 hours. Subsequently, media were discarded and 100 µL of lysis solution (SDS10 % in HCl 0.01 N) were added to the wells and left overnight at 37 °C. Absorbance was measured at 540 nm.

Adipose stromal cells (ASCs) adipogenic differentiation

ASCs were seeded in CM_{ASC} and incubated for 48 hours at 37 °C in a humidified atmosphere at 5 % CO_2 and 19 % O_2 to permit cell adhesion. Adipogenic differentiation was performed by exposing cells to 3 cycles of 4 days each, of the following treatments:

- 3 days with induction medium: CM_{ASC} supplemented with dexamethasone (DEX) at 1 µM, insulin (INS) at 1.7 µM, 3-iso-butyl-1-methylxanthine (IBMX) at 0.5 mM and indomethacin at 250 µM.
- 1 day with differentiation maintaining medium: CM_{ASC} supplemented with insulin (INS) at 1.7

μM.
Negative controls were performed using ASCs cultured in CM_{ASC}. Cell differentiation was evaluated by Oil Red O Staining to detect cytoplasmic lipid vacuoles as markers of adipogenesis.

Expression of adipogenic marker genes
In order to evaluate the expression of adipogenic marker genes crucial for the function of adipose tissue, PPARγ and Leptin total RNA was extracted from ASCs pellets (7×10^5 cells), subjected or not to adipogenic differentiation, using NucleoSpin RNA II. cDNA was then obtained by reverse transcription of 2 μg of the extracted RNA using the High-capacity cDNA reverse transcription kit. The expression of the genes was evaluated by PCR (Polymerase Chain Reaction) using the primers for PPARγ, leptin and GADPH. The occurrence of the amplification was verified by 2.5% agarose gel electrophoresis in TAE buffer (Tris base, glacial acetic acid, EDTA 0,5 M, pH 8), where the correct length of the amplicons was verified by comparison with Gene Ruler™ 100 bp DNA LaddeR markers. DEPC treated water was used instead of cDNA as a negative control for all reactions. The DNA patterns were displayed under UV light and the images were captured with a PowerShot A610 Canon camera.

Solubilisation and quantification of Oil Red O production
To evaluate intracellular lipid accumulation, 20×10^4 ASCs were plated in 24 wells plates and subjected to a differentiation process with glyphosate at 0 or 4 μg/mL. After 12 days the cells were washed 3 times with PBS, fixed with 4% formaldehyde for 1 hour and washed with 60% isopropanol. The cells were then dried and stained with Oil Red O staining solution for 30 minutes. After multiple washings, Oil Red O was solubilized in each well with 60% isopropanol for 2 hours and the absorbance measured at 540 nm. Absorbance was considered to be proportional to the amount of differentiated cells.

Adipose cell counting
5×10^4 MSCs were seeded on a cover slip in 6-well plates and subjected to the adipose differentiation process with glyphosate at 0 or 4 μg/mL. After 12 days, cover slips were fixed with formalin (4%) and stained with Oil Red O. Then, cells were stained in Mayer haematoxylin for 3 minutes and images made by light microscopy (total magnification 10x) and quantified by counting the stained cells in 10 different fields.

Statistical analysis
The experiments were performed five times on granulosa cells and three times on ASCs and six replicate wells were used for the assessment of the effect of glyphosate. Every time the adipose tissue was collected from 3 pigs. Data are presented as the mean ± SEM. Statistical differences were calculated by ANOVA using Statgraphics software. In the presence of a significant difference ($p < 0.05$), the means were subjected to Scheffè' F test for multiple comparisons.

II. RESULTS AND DISCUSSION

The effect of glyphosate on swine granulosa cells
Glyphosate statistically significantly decreased cell proliferation ($p < 0.001$) as evaluated by BrdU incorporation (see Figure 1) and cell viability ($p < 0.05$) as measured by ATP production (see Figure 2)
without a concentration-response relationship. Granulosa cell steroidogenesis was affected by glyphosate. E2 secretion was statistically significantly inhibited ($p < 0.05$, Figure 3a) whereas P4 secretion was statistically significantly increased ($p < 0.05$, Figure 3b) both at all tested concentrations but without a concentration-response relationship. While O_2^--production was not statistically significantly modified by glyphosate at any of the concentrations tested, NO production was significantly stimulated ($p < 0.001$, Figure 4) at all the tested concentrations although with no statistically significant differences amongst them. Scavenging activity, represented by non-enzymatic antioxidant power, was significantly inhibited ($p < 0.05$, Figure 5) by glyphosate at all the concentrations tested but also without significant differences among them.

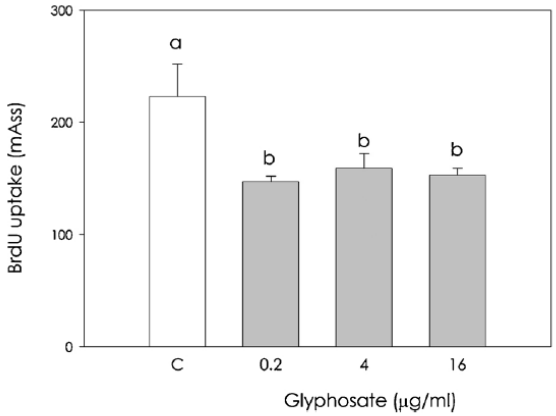


Fig. 1. Effect of the treatment with glyphosate (0.2, 4 and 16 µg/ml) for 48 h on swine granulosa cell proliferation using 5-bromo-2′-deoxyuridine (BrdU) incorporation assay test. Data, expressed as milliAss units, represent the mean ± SEM of six replicates/treatment repeated in five different experiments. Different letters on the bars indicate a significant difference ($P < 0.001$) among treatments as calculated by ANOVA and Scheffè' F test.

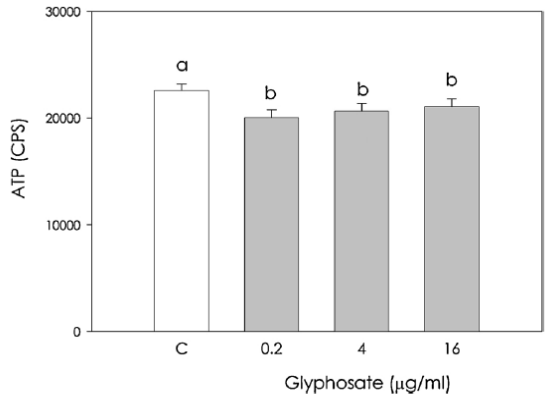


Fig. 2. Effect of the treatment with glyphosate (0.2, 4 and 16 µg/ml) for 48 h on swine granulosa cell viability using ATP content assay test. Data, expressed as counts per second (CPS), represent the mean ± SEM of six replicates/treatment repeated in five different experiments. Different letters on the bars indicate a significant difference ($P < 0.05$) among treatments as calculated by ANOVA and Scheffè' F test.

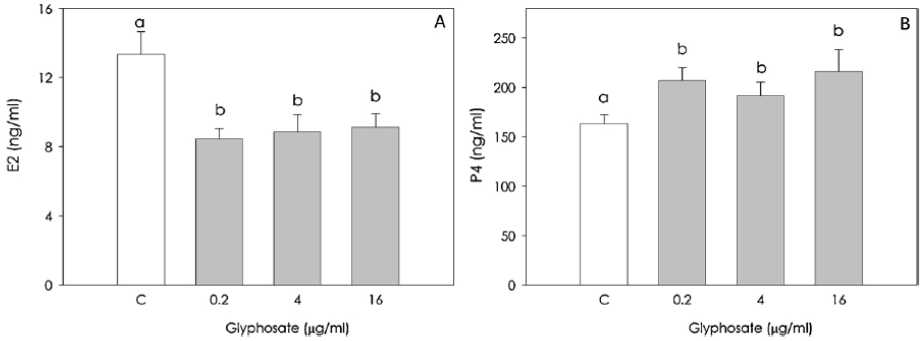


Fig. 3. Effect of the treatment with glyphosate (0.2, 4 and 16 µg/ml) for 48 h on swine granulosa cell estradiol 17β (E2) production (A) or progesterone production (B) using RadioImmunoAssay (RIA). Data, expressed as ng/ml, represent the mean ± SEM of six replicates/treatment repeated in five different experiments. Different letters on the bars indicate a significant difference ($P < 0.05$) among treatments as calculated by ANOVA and Scheffè' F test.

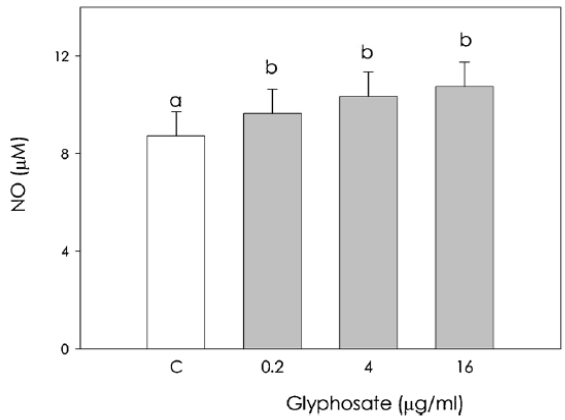


Fig. 4. Effect of the treatment with glyphosate (0.2, 4 and 16 µg/ml) for 48 h on swine granulosa cell nitric oxide (NO) production using Griess Assay. Data, expressed as µM, represent the mean ± SEM of six replicates/treatment repeated in five different experiments. Different letters on the bars indicate a significant difference ($P < 0.001$) among treatments as calculated by ANOVA and Scheffè' F test.

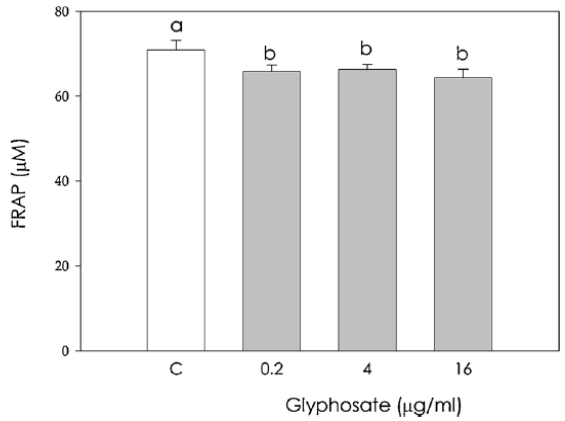


Fig. 5. Effect of the treatment with glyphosate (0.2, 4 and 16 µg/ml) for 48 h on swine granulosa cell non-enzymatic scavenging activity by swine granulosa cells using the FRAP assay. Data, expressed as µM, represent the mean ± SEM of six replicates/treatment repeated in five different experiments. Different letters on the bars indicate a significant difference ($P < 0.05$) among treatments as calculated by ANOVA and Scheffè' F test.

The effect of glyphosate on swine adipose stromal cells (ASCs)

The histological analysis of the isolated fragments obtained from pig fat showed typical characteristics of adipose tissue (figure 6, panel I a, b). In particular, adipocytes containing a single large lipid droplet with thin cytoplasm and flattened peripheral nucleus are surrounded by a microvasculature dispersed in the stromal tissue.

After 3 to 4 days of *in vitro* culture of the fragmented explants, there was a migration of cells characterized by typical mesenchymal fibroblast-like morphology, which proliferated in adhesion to the bottom of the well (Figure 6, panel II a, b).

After 5 to 7 days from fragment removal, ASCs reached 80% confluence in monolayer, maintaining their morphological characteristics (Figure 6 panel III a,b). The proliferation of ASCs under basic culture conditions was statistically significantly increased ($p < 0.001$) (454 ± 12 vs 476 ± 7), while glyphosate treatment at 4 µg/mL significantly decreased ($p < 0.001$) the viability of proliferating ASCs both after 48 (432 ± 10) and 72 hours (451 ± 7).

The expression of PPARγ and LEP adipogenic markers was not observed in undifferentiated ASCs, but was detected following adipogenic differentiation (Figure 7).

Adipogenic differentiation of ASCs was achieved, as shown by the appearance of red lipid droplets in the differentiated cell cytoplasm. Differentiated cell counts showed a significant inhibition ($p < 0.05$) of the adipogenic process by glyphosate at 4 µg/mL. These data were confirmed by spectrophotometric evaluation of solubilized Oil RedO.

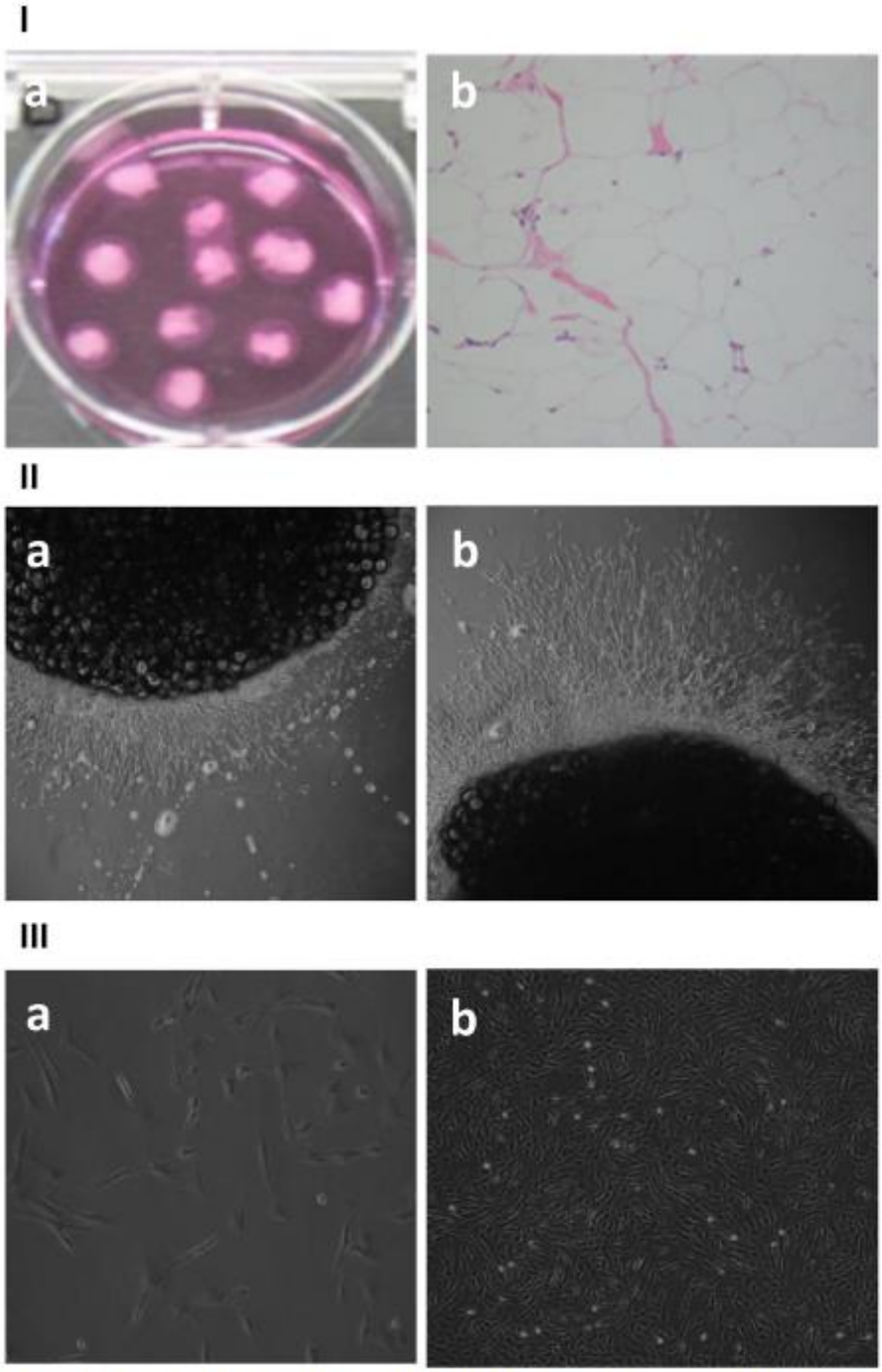


Fig. 6. Panel I: fragments of adipose tissue in culture (a) and histological section (b)(ematoxylin and eosin 20X). Panel II: representative images of adipose tissue in culture showing ASCs attachment and proliferation. Panel III: cultured ASCs. a: fibroblast-like morphological aspect (4X). b: 80% of confluence (10X).

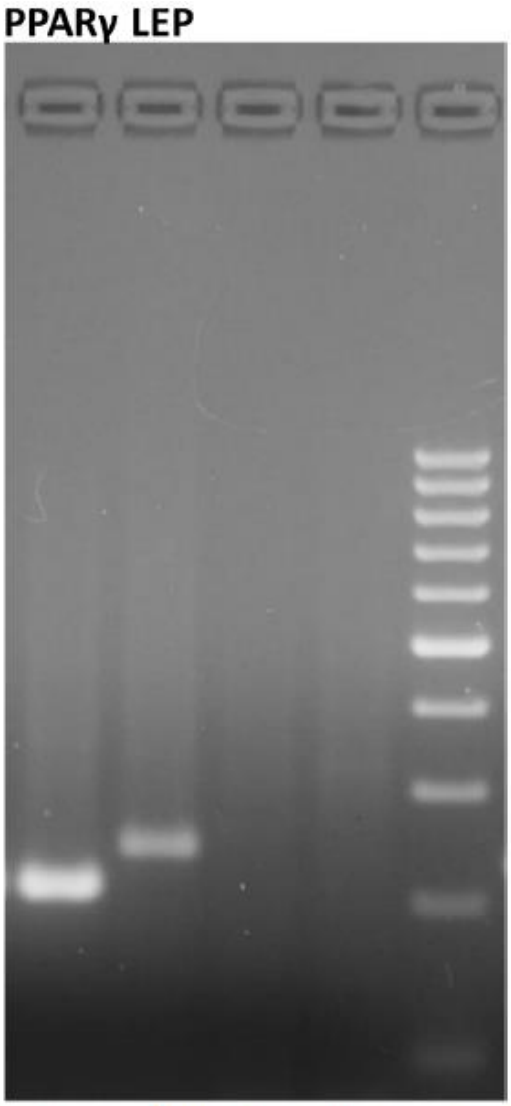


Fig. 7. Peroxisome proliferator-activated receptor γ (PPARγ) and leptin (LEP) expression in ASCs after adipogenic differentiation.

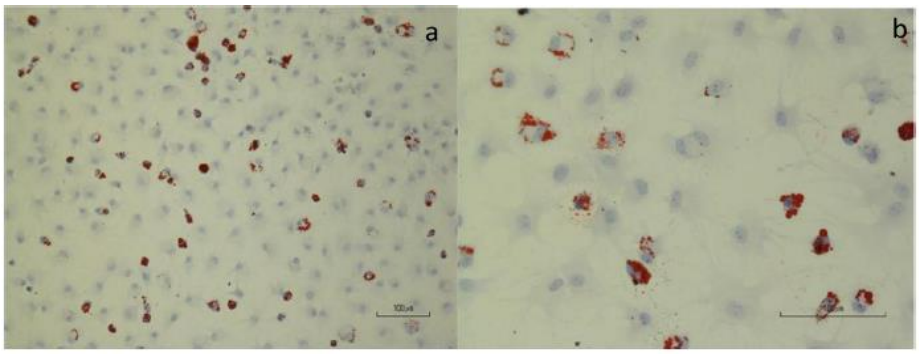


Fig. 8. Panel I: spectrophotometric quantification (mAss) after Oil Red O staining of ASCs. Panel II: optical microscope images of ASCs undergoing adipogenic differentiation after Oil Red O staining. a): 10×; b): 20×.

Discussion

This study shows that glyphosate is able to significantly stimulate P4 production. This is a critical effect for granulosa cells because P4 is a hallmark of luteinization. Ovarian steroidogenesis is a key function for the survival of GCs, and is essential for follicular development and growth. To verify the potential effects of glyphosate on follicular physiology, the present study also analyzed the possible effect of glyphosate on granulosa cell proliferation and metabolic activity. The data show an inhibition of DNA replication activity and

ATP production at all concentrations analyzed suggesting a disruption of follicular development in vivo.
Since endothelial cell characteristics have been recently attributed to granulosa cells, it is interesting to evaluate the increased NO production in granulosa cells following glyphosate exposure. It can be hypothesized that glyphosate exerts direct pro-angiogenic effects on pigs GCs at all tested concentrations as a result of a significant increase in NO, which is a key molecule of this process. The stimulation of NO production can also be associated with an increase in oxidative stress which seems to be supported by the glyphosate-induced reduction of the non-enzymatic antioxidant power in GCs at all concentrations tested. Adipose stromal cells (ASCs) were isolated from abdominal subcutaneous fat tissue of pig and characterized. Their ability to differentiate into adipocytes under appropriate stimuli has been evaluated on the basis of the expression of PPARγ and leptin genes that was not detected in undifferentiated ASCs. These results were essential to define an adequate experimental model to investigate potential effects of glyphosate on the expression of these adipogenic markers. The inhibitory effect of glyphosate during induction of ASCs adipogenic differentiation suggests that this substance, once accumulated as a consequence of environmental exposure, can interfere with adipose tissue biology *in vivo*. Moreover, glyphosate was found to significantly decrease the viability of ASCs after 48 and 72 hours of exposure. Such evidence appears to be critical since it suggests a direct interference of glyphosate with adipose tissue function.

Study conclusion
In this *in vitro* study it is shown that glyphosate inhibits cell growth, 17-β estradiol production and non-enzymatic scavenging activity and increased progesterone production and nitric oxide secretion in granulosa cells. In addition, glyphosate significantly decreased the viability of adipose stromal cells (ASCs) and inhibited their adipogenic differentiation. These data are indicative of the interference of glyphosate with the main functional parameters of granulosa cells and ASCs and could affect both reproductive function and adipogenic processes *in vivo*.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of glyphosate on functional parameters of granulosa cells and adipose stromal cells from swine were investigated *in vitro*. In granulosa cells the effect of glyphosate was studied on cell proliferation, cell viability, steroid production, superoxide anion production, NO production and ferric reducing activity. In adipose stromal cells the effect of glyphosate was studied on cell viability, adipogenic differentiation, adipogenic marker genes (PPARγ and leptin), intracellular lipid accumulation and adipose cell count. Glyphosate was found to significantly decrease cell proliferation, cell viability, estrogen production and ferric reducing capacity and increase progesterone and NO production in granulosa cells when tested at concentrations ranging from 0.2 to 16 µg/mL. However, in none of the assays with granulosa cells a concentration-response relationship was established. Glyphosate treatment at 4 µg/mL significantly decreased ($p < 0.001$) the viability of proliferating adipose stromal cells after 48 and 72 hours. Differentiated cell counts showed a significant inhibition ($p < 0.05$) of the adipogenic process by glyphosate at 4 µg/mL. Since only one concentration of glyphosate was tested it was not possible to establish a concentration-response relationship. In this publication it is suggested that glyphosate interferes with the main functional parameters of the granulosa cell which could affect reproductive function. No effects on female reproductive function were reported in the rat in regulatory reproductive toxicology tests at doses beyond 2,000 mg/kg bw/day producing systemic glyphosate concentrations that are higher than those tested in this study.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized, no positive controls were included in the assays and only one dose level was used for the testing of adipose stromal cells.

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

Publication: Gigante *et al.*, 2018.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	

Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity is not reported. Source: Sigma Chemical Co, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Granulosa cells from swine ovaries, adipose stromal cells from swine adipose tissue.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.2, 4 or 16 µg/mL.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive control.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized, no positive controls were included in the assays and only one dose level was used for the testing of adipose stromal cells.		

Assessment and conclusion by RMS:

The key elements of the material and method and results section are adequately described. Unlike the applicant the RMS does not consider that the lack of a positive control affects the reliability of the study. Overall, the study is concluded to be reliable (Klimisch score 1).

The results of the study give an indication of a potential effect of glyphosate on functional parameters of granulosa cells and ASCS. However, the results from this *in vitro* cannot be directly translated to an adverse *in vivo* outcome due to the uncertainty of how the *in vitro* concentrations reflect *in vivo* doses.

B.6.8.3.12. Public literature – Effect of glyphosate on the TM4 Sertoli cell line

Data point:	CA 5.8.3
Report author	Vanlaeys, A. *et al.*
Report year	2018
Report title	Formulants of glyphosate-based herbicides have more deleterious impact than glyphosate on TM4 Sertoli cells
Document No	doi.org/10.1016/j.tiv.2018.01.002 E-ISSN: 1879-3177
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	No

GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable (Klimisch score 1)

Introduction
Roundup and Glyphogan are glyphosate-based herbicides containing the same concentration of glyphosate and confidential formulants. Formulants are declared as inert diluents but some are more toxic than glyphosate, such as the family of polyethoxylated alkylamines (POEA). In this study glyphosate alone, glyphosate-based herbicide formulations and POEA on the immature mouse Sertoli cell line (TM4) was tested, at concentrations ranging from environmental to agricultural-use levels.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material
Glyphosate was purchased from Sigma-Aldrich, St Louis, USA. The purity was not reported.
The formulation tested were Glyphogan and Roundup Bioforce.

Culture of TM4 Sertoli cells and treatments
The murine TM4 Sertoli cell line was obtained from the American Type Culture Collection (ATCC Manassas, USA). Cells were maintained in DMEM/HamF12 medium containing 0.2 % glutamine, 1.2 g/L $NaHCO_3$, 15mM Hepes, 5 % horse serum and 2.5 % foetal calf serum, 100 U/mL of antibiotics and fungizone (complemented DMEM/Ham F12 medium) at 37 °C (5 % CO2, 95 % air) during 24 hours to 80 % confluence in 24-well plates or in 6-well plates (for measurement of GST activity). Cells were then exposed to various concentrations of glyphosate alone or in the commercial formulation Roundup Bioforce of Glyphogan.

B. STUDY DESIGN

Crystal violet cell viability assay
After incubation with glyphosate at different concentrations, culture supernatants were discarded and cells incubated in medium containing crystal violet solution (0.1 % w/v in PBS 0.01 M, pH 7.4) for 30 minutes at 20 °C with gentle rocking. Excess dye and non-adherent dead cells were removed through 3 washing steps with PBS. Diluted acetic acid solution (10 %) was then added to release the crystal violet taken up by cells, and the optical density reflecting living adherent cells was determined by absorption at 600 nm using a plate reader.

MTT cytotoxicity assay
This enzymatic test is based on the cleavage of MTT (tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a blue-colored product (formazan) by the mitochondrial enzyme succinate dehydrogenase. Activity of mitochondrial dehydrogenase enzymes indirectly measures activity of mitochondrial respiration and antioxidant defense systems. Culture medium was removed, and cells were washed once with PBS and then incubated with 500 µL MTT solution per well after each treatment. The plates were incubated for 3 hours at 37 °C. The reaction was stopped by placing the plates for 10 minutes at 4 °C followed by adding to each well 500 µL 0.04 N hydrochloric acid-containing isopropanol. The plates were then vigorously shaken for 40 min to solubilize the blue formazan crystals formed. Absorbance was measured by spectrophotometry at 570 nm.

Glutathione-S-transferase (GST) activity
For preparation of S9 fractions enriched in GST, the medium was removed, and cells were detached by treatment with trypsin-EDTA and washed twice with PBS at room temperature. Cells were then resuspended in 500 µL of 50 mM phosphate buffer at pH 7.2 containing 0.25 M sucrose and 1mM DTT. Then, they were homogenized and centrifuged at 9000g at 4 °C for 30 minutes. Supernatants corresponding to the S9 fraction were collected and stored at -80 °C. The protein concentration of each S9 fraction was determined using the Bradford assay. For the determination of GST activity 250 µg of S9 cell fraction was mixed with 10 µL of 100 mM reduced L-glutathione and 990 µL phosphate buffer (pH 6.5). The reaction was initiated by the addition of 10 µL of 100

mM 1-chloro-2,4-dinitrobenzene (CDNB) solution in 95% ethanol. After incubation for 90 seconds at 37 °C absorbance was measured at 340 nm every 60 seconds for 3 minutes using the spectrophotometer.

Lipid Sudan Black B staining
Sudan Black B stains lipids, including phospholipids and sterols. Culture medium was removed and cells were washed once with PBS. Cells were then incubated for 5 minutes at room temperature with 500 µL of Sudan Black solution with gentle shaking. This solution was removed, and cells were washed 3 times or more with 70 % ethanol to remove excess stain and then at least 3 times with PBS. Then, intracellular Sudan Black B was extracted by incubation with DMSO for 30 minutes with gentle rocking and absorbance measured at 600 nm using a microplate reader.

Statistical analysis
The experiments were repeated at least in triplicate in different weeks on three independent cultures on each occasion (n=9). All data are presented as the mean ± standard error (SEM). Statistical differences from controls were determined by an ANOVA test. Results were statistically significantly different from controls when $p < 0.05$.

II. RESULTS AND DISCUSSION

Viability of TM4 cells
Glyphosate was found to have no impact on cell viability of TM4 cells after 24 hours of exposure at concentrations ranging from 10 to 10,000 ppm. In contrast, glyphosate based formulation induced dose-dependent cell death.

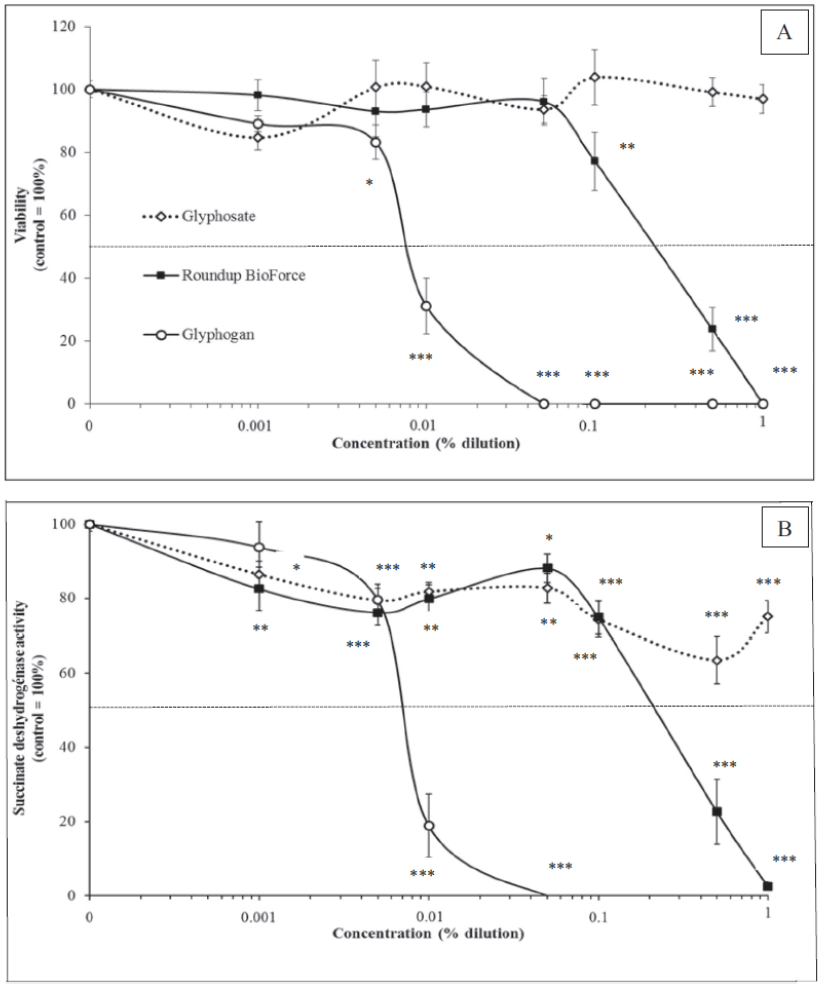


Fig. 1. Effects of Roundup, Glyphogan or Glyphosate on TM4 viability after 24 h of treatment. Cells were grown at 37 °C (% CO_2, 95% air) in complemented DMEM/Ham F12 medium (cDH) for 24 h until 80% confluent. Then, cells were exposed to different dilutions of glyphosate formulations (Roundup Bioforce® or Glyphogan) or equivalent doses of Glyphosate in cDH for 24 h. Cytotoxicity of Roundup Bioforce®, Glyphogan or Glyphosate alone were evaluated using the Crystal violet (A) and MTT (B) assays. A value of 0% of succinate dehydrogenase activity reveals total cell death (B). SEM are shown in all instances (Anova test $p < 0.05^{*}$, $p < 0.01^{**}$ and $p < 0.001^{***}$). The LC50 of Roundup Bioforce and Glyphogan are indicated by the empty square above the curves.

Mitochondrial succinate dehydrogenase activity

The measurement of succinate dehydrogenase (SD) activity was used to assess the effect of glyphosate on mitochondrial function and viability after 24 hours of exposure. Reduced SD activity was seen for glyphosate over the entire concentration range from 0.001% (approx. 85%) to 1% (approx. 75%) as derived from the graph. No clear dose-response relationship was observed. The two glyphosate based formulation resulted in a stronger reduction of SD activity.

Inhibition of glutathione-S-transferase (GST) activity

The effect of glyphosate on GST activity involved in the anti-xenobiotic defense system was evaluated. At an LC_{50} concentration glyphosate was found to have no impact on GST activity. Glyphosate based formulations inhibited GST activity (40%).

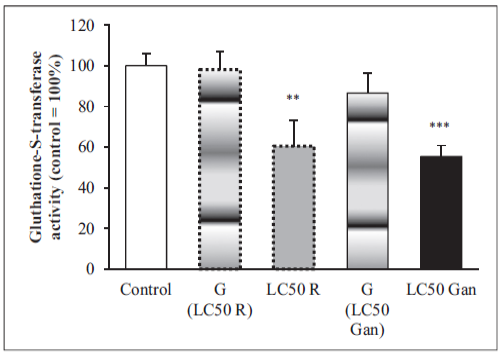


Fig. 2. Effects of Glyphosate (G) alone or in herbicide formulations (Bioforce (R) or Glyphogan (Gan)) on TM4 cell Glutathione-S-transferase activity after 24 h exposure. Cells are treated at LC50 concentrations of formulations (0.22% R, 0.0075% Gan) or equivalent doses of G. GST activity was evaluated in S9 cell fractions. SEM are shown in all instances (Anova test $p < 0.05^{*}$, $p < 0.01^{**}$ and $p < 0.001^{***}$). For more details see the caption of Fig. 1.

Lipid droplet accumulation

After staining with Sudan Black B exposure of TM4 cells to glyphosate for 24 hours at 2,500 or 5,000 ppm induces an increase in cytoplasmic lipid droplets as assessed microscopically. This accumulation was greater after treatment with a formulation.

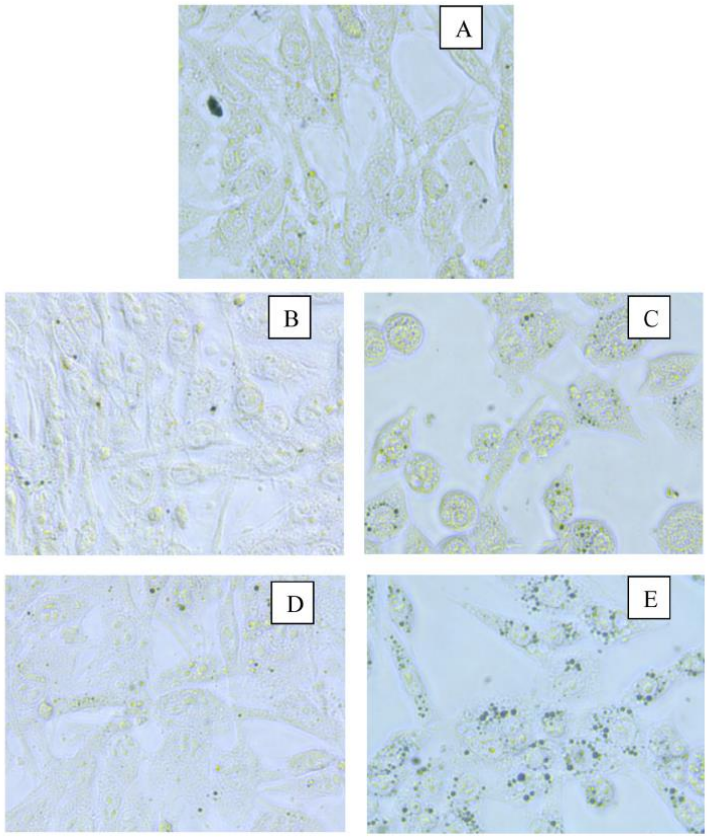


Fig. 4. TM4 cell lipid droplet assessment after 24 h treatment with Roundup Bioforce or Glyphosate alone. At 80% confluence, cells were exposed to various concentrations of Roundup Bioforce® or equivalent doses of Glyphosate for 24 h (see Fig. 1.). After Sudan Black B staining, lipid droplets appear black (200×) in control cells (A); cells treated with glyphosate 0.25% (B) or 0.5% (D), with Roundup Bioforce 0.25% (C) or 0.5% (E).

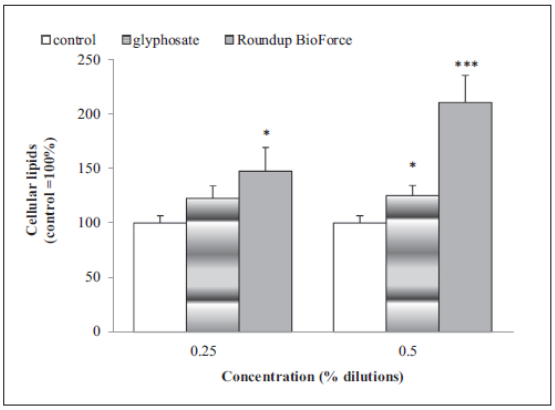


Fig. 5. Effects of Glyphosate alone or in Roundup Bioforce formulation on TM4 cell lipid droplet accumulation after 24 h exposure. Cell cultures, treatments and lipid staining were performed as described in Figs. 1 and 5. After lipid Sudan Black B extraction, quantity of stain was evaluated by spectrophotometric analysis at 600 nm. SEM are shown in all instances (Anova test $p < 0.05^{*}$, $p < 0.01^{**}$ and $p < 0.001^{***}$).

Discussion

Measurements of cell viability, respiratory chain activity, detoxification system and lipid accumulation were undertaken in immature murine TM4 Sertoli cell line following 24 hours of exposure to glyphosate at concentrations ranging from environmental levels to agricultural use concentrations (1%, 10,000 ppm). This study demonstrated that at sub-agricultural use levels (10-10,000 ppm) TM4 cell viability is not affected by glyphosate alone but was affected by glyphosate formulations. Exposure of TM4 cells to glyphosate reduces mitochondrial succinate dehydrogenase (SD) activity at the lowest concentrations tested in TM4 cells, as compared with other cell types, with a greater cytotoxic impact seen with formulations. The formulants present in commercial herbicides are known to increase glyphosate penetration into cells by membrane disruption and thus probably potentiate perturbation of mitochondrial permeability induced by glyphosate. This mechanism may explain the higher toxicity of formulations on mitochondrial activity. In this study, it was also demonstrated that 24 hours exposure of TM4 cells to glyphosate induced lipid droplet accumulation.

III. CONCLUSIONS

Assessment and conclusion by applicant:

In this study the effect of glyphosate on murine TM4 Sertoli cells was investigated *in vitro*. The endpoints were cytotoxicity, glutathione transferase activity and lipid accumulation. In contrast to the glyphosate-based formulations and co-formulants tested glyphosate was found to have no impact on cell viability after 24 hours of exposure at concentrations ranging from 10 ppm to 10,000 ppm. Glyphosate reduced succinate dehydrogenase to some extent over the entire concentration range from 10 (approx. 85 % of control) to 10,000 ppm (approx.75 % of control) with no dose-effect relationship and was found to have no impact on glutathione transferase activity. Exposure of TM4 cells to glyphosate for 24 hours at 2,500 or 5,000 ppm induces an increase in cytoplasmic lipid droplets. These concentrations are far beyond what is physiologically feasible *in vivo*.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used is not sufficiently characterized and no positive controls were used in any of the assays conducted.

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

Publication: Vanlaeys *et al.*, 2018	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically	Y?	

acceptable standards		
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also co-formulants and formulations were tested.
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	
Test concentrations in physiologically acceptable range (<1 mM)	Y (partly)	
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive controls used.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used is not sufficiently characterized and no positive controls were used in any of the assays conducted.		

Assessment and conclusion by RMS:

The key elements of the material and method and results section are adequately described. Unlike the applicant the RMS does not consider that the lack of a positive control affects the reliability of the study. Overall, the study is concluded to be reliable (Klimisch score 1).

The study showed that glyphosate on its own had little to no effect on TM4 Sertoli cells while glyphosate based formulations did.

B.6.8.3.13. Public literature – Effect on ER-mediated cell proliferation

Data point:	CA 5.8.3
Report author	Mesnage, R. *et al.*
Report year	2017
Report title	Evaluation of estrogen receptor alpha activation by glyphosate-based herbicide constituents
Document No	doi.org/10.1016/j fct.2017.07.025 E-ISSN: 1873-6351
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing	Not applicable

facilities	
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable without restrictions Conclusion AGG: Reliable (Klimisch Score 1)

Executive Summary
In this study, the estrogenic potential of glyphosate, commercial GBHs and polyethoxylated tallowamine adjuvants present as co-formulants in GBHs was evaluated. Glyphosate (≥10,000 µg/L or 59 µM) promoted proliferation of estrogen-dependent MCF-7 human breast cancer cells. Glyphosate also increased the expression of an estrogen response element-luciferase reporter gene (ERE-luc) in T47DKBluc cells, which was blocked by the estrogen antagonist ICI 182,780. Commercial GBH formulations or their adjuvants alone did not exhibit estrogenic effects in either assay. Transcriptomics analysis of MCF-7 cells treated with glyphosate revealed changes in gene expression reflective of hormone-induced cell proliferation but did not overlap with an ERα gene expression biomarker. Calculation of glyphosate binding energy to ERα predicts a weak and unstable interaction (-4.10 CAl mol-1) compared to estradiol (-25.79 CAl mol-1), which suggests that activation of this receptor by glyphosate is via a ligand-independent mechanism. Induction of ERE-luc expression by the PKA signaling activator IBMX shows that ERE-luc is responsive to ligand-independent activation, suggesting a possible mechanism of glyphosate-mediated activation. The authors conclude that the study reveal that glyphosate, but not other components present in GBH formulations can active ERα *in vitro*, albeit at relatively high concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material
Glyphosate used was the PESTANAL® analytical standard (≥98.0%) obtained from Sigma-Aldrich (UK). The batch of glyphosate (≥98.0%) purchased from AccuStandard (New Haven, CT, USA) was tested exclusively in the ERE transcription luciferase reporter gene assay. Glyphosate-based formulations available on the market were Glyphogan (France, 39-43% iso-propylamine salt of glyphosate, 13-18% of POEA), Roundup Grand Travaux Plus (France, 450 g/L of glyphosate, 90 g/L of ethoxylated etheralkylamine), Roundup Original DI (Brazil, 445 g/L of glyphosate diammonium salt, 751 g/L of other ingredients) and Roundup Probio (UK, 441 g/L of the potassium salt of glyphosate, and other ingredients). POEA was purchased from ChemService (West Chester, PA, USA). The agricultural spray adjuvant was Gen-amin T200 (France, 60-80% of POE-15).

Cell culture
MCF-7, MDA-MB-231 and T47D cell lines were obtained from Prof Joy Burchell (Research Oncology Department, King's College London). T47D-KBluc cells were purchased from the American Type Culture Collection (ATCC, Teddington, UK) and harbour a stably integrated copy of a luciferase reporter gene under control of a promoter containing ERE. All cells were grown in a maintenance medium. Stock solutions of glyphosate, glyphosate-based herbicide formulations, POEA and Genamin T200 surfactant formulation were prepared in serum-free medium and adjusted to pH 7.2. Stock solutions of with estradiol, 3-isobutyl-1-methylxanthine (IBMX) and BPA were prepared by dissolution in ethanol. The solutions for testing were prepared by dilution of the stock solutions in test medium taking care that the solvent concentrations were always kept below 0.5% for the cell assays and below 0.0005% for transcriptome profiling. Cells were released from the flask substrate with trypsin and counted with a hemocytometer prior to seeding. A 24-hour recovery period was allowed for cell adherence in DMEM maintenance medium before cultures were subjected to the desired tests.

B. STUDY DESIGN

E-screen assay
The E-screen allows the determination of estrogenic effects by measuring ER-mediated cell proliferation in hormone responsive cells (MCF-7, MDA-MB-231, T47D). Cells were seeded into 48-well plates at a density of 20,000 cells per well in 250 µL maintenance medium. Following a 24-hour incubation to allow cell attachment, the medium was changed to the medium containing the test compounds. The test medium was refreshed after 3 days. Following another 3-day period of incubation, an MTT assay was performed. The MTT test allows the

measurement of cytotoxic effects since the activity of mitochondrial dehydrogenase enzymes indirectly reflects cellular mitochondrial respiration. Cells were incubated with 250 µL of MTT solution for 2 hours. The test was terminated by lysing the cells with dimethyl sulfoxide (DMSO) and optical density was measured at 570 nm using the SPECTROstar Nano plate reader. The proliferative effect was expressed as a percentage of the control cell culture receiving no treatment.

ERE-luciferase reporter gene assay
The ERE-mediated transcription of a luciferase reporter gene was determined in T47D-KBluc cells using the Steady-Glo® luciferase assay system following the manufacturer's instructions. T47D-KBluc cells were seeded in 96-well plates at a density of 20,000 cells per well in 50 µL of maintenance medium and allowed to attach overnight. Prior to adding the test substance an initial 24-hour incubation was performed in the absence of test substance to improve residual estrogen clearance and assay sensitivity. After incubation, Steady-Glo® luciferase reagent was added. The plates were left to stand for 10 minutes in the dark at room temperature to allow cell lysis. Bioluminescence was measured using the Orion II microplate luminometer. ER-mediated gene activation was confirmed by ascertaining if the observed effects were subject to inhibition by addition of the estrogen antagonist ICI 182,780.

Microarray gene expression profiling
MCF-7 cells were seeded into 96-well plates with maintenance medium at a density of 20,000 cells per well. After steroid deprivation in hormone free medium, the cells were treated with test substance in triplicate in three independent experiments. RNA extraction was performed using the Agencourt RNAdvance Cell V2 kit according to the manufacturer's instructions. The samples were checked for RNA quality and quantified. Subsequently, technical replicates of samples, were pooled appropriately such that the final input amount of each biological replicate was 3 ng. Transcriptome gene expression profiles were determined using the Affymetrix Human Transcriptome 2.0 Array. Data were imported and normalized together in Omics Explorer 3.0 using the Robust Multi-array Average (RMA) sketch algorithm. These microarray data were submitted to Gene Expression Omnibus (NCBI) and are accessible through accession number GSE86472.

RNA-sequencing gene expression profiling
Gene expression profiling was performed by applying Illumina sequencing by synthesis technology. The RNA-seq data were submitted to Gene Expression Omnibus (NCBI) and are accessible through accession number GSE8770.

Statistical analysis
The ERE transcription luciferase reporter gene assay and the E-Screen assay were performed 4 and 6 times in triplicate, respectively. The concentrations required to elicit a 50% response (AC_{50}) were determined using a nonlinear regression fit. For the transcriptome analysis, pair-wise comparisons were performed using a t-test controlling for batch effects in Omics Explorer 3.0. Affymetrix microarray and RNA-seq gene expression profiling were performed 3 times in triplicate. Data used for the functional analysis were selected at cut off p-values of <0.05 with fold change >1.2 to evaluate the ER activation signature. Gene and disease ontology were analyzed using the Thomson Reuters MetaCore Analytical Suite recognizing network objects (proteins, protein complexes or groups, peptides, RNA species, compounds among others). The p-values are determined by hypergeometric calculation and adjusted using the method of Benjamini and Hochberg.

Molecular dynamics simulation and ONIOM binding energy calculations
The atomic coordinates of the complex formed by estradiol (ES) and the human ERa ligand binding domain were taken from the Protein Data Bank (entry code 1A52). For the construction of the model, only chain A of the homodimer was included, and the second set of duplicate coordinates was deleted. Glyphosate was docked into the ER by using the ArgusLab software.

II. RESULTS AND DISCUSSION

E-screen assay
The AC_{50} of 17β-estradiol and BPA in MCF-7 human breast cancer cells was 0.0013 µg/L and 46 µg/L, respectively. Glyphosate induced cell proliferation starting between 1,000 and 10,000 µg/L and peaking around 1,000,000 µg/L. Similar but less pronounced results were observed with the T47D cell line with cells retaining a response to glyphosate but not to Roundup Probio. No proliferative effects were observed in the ER-negative, hormone-independent MDA-MB-231 cell line, suggesting that the proliferative effects were mediated via the ER. Roundup Probio, tested in MCF-7 cells induced a non-statistically significant trend of cell proliferation.

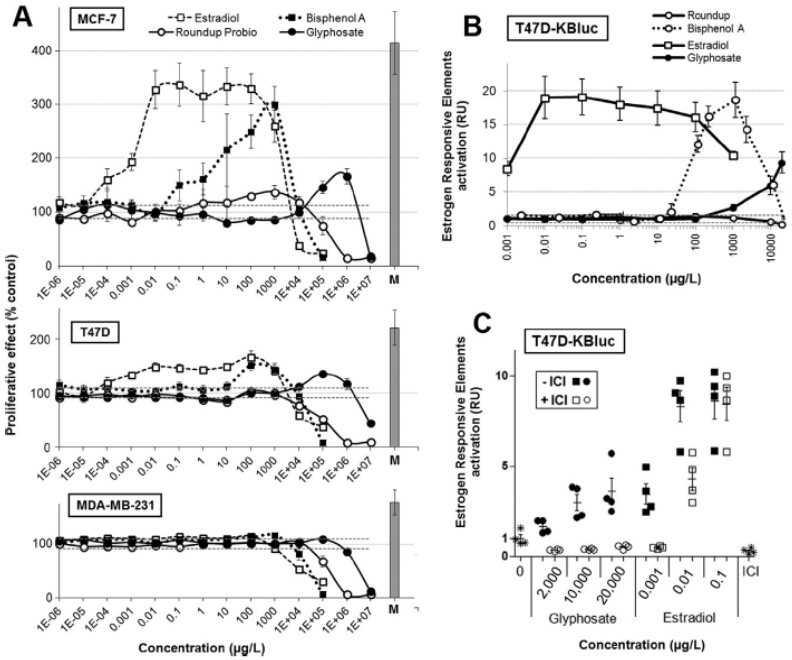


Fig. 1. Glyphosate at high concentrations can substitute for estradiol in promoting cell growth through estrogen receptors in human breast cancer cells. (**A**) Proliferative effect of glyphosate and Roundup Probio in an E-Screen bioassay. Hormone-dependent (MCF-7, T47D) and hormone independent (MDA-MB-231) human breast cancer cells were cultured under hormone-free conditions (n = 6). Proliferative effects by MTT assay following 6 days exposure to either glyphosate or Roundup Probio with estradiol and bisphenol A treatments acting as positive controls at the indicated concentrations. Proliferation is expressed as a percentage increase in cell number compared to culture in the absence of the natural hormone or test substance. Upper bound and lower bound of the biological variability (SEM) of the non-treated cells (negative control) are represented by dotted lines. M, proliferative effect of medium containing non-steroid hormone stripped FBS. (**B**) Estrogen receptor activation in T47D-Kluc cells harbouring a ERE-luciferase reporter gene. Cells were treated for 24 h with glyphosate alone, a Roundup formulation (Roundup Probio), BPA or estradiol and activation of expression measured by a bioluminescence assay (n = 4). (**C**) Luciferase assays of T47D-Kluc cell cultures treated with glyphosate or estrodiol in the absence (black squares, circles) or presence (white squares, circles) of the estrogen antagonist ICI 182,780 (n = 4). Note decrease in luciferase reporter gene expression indicating action of test substances via the ER.

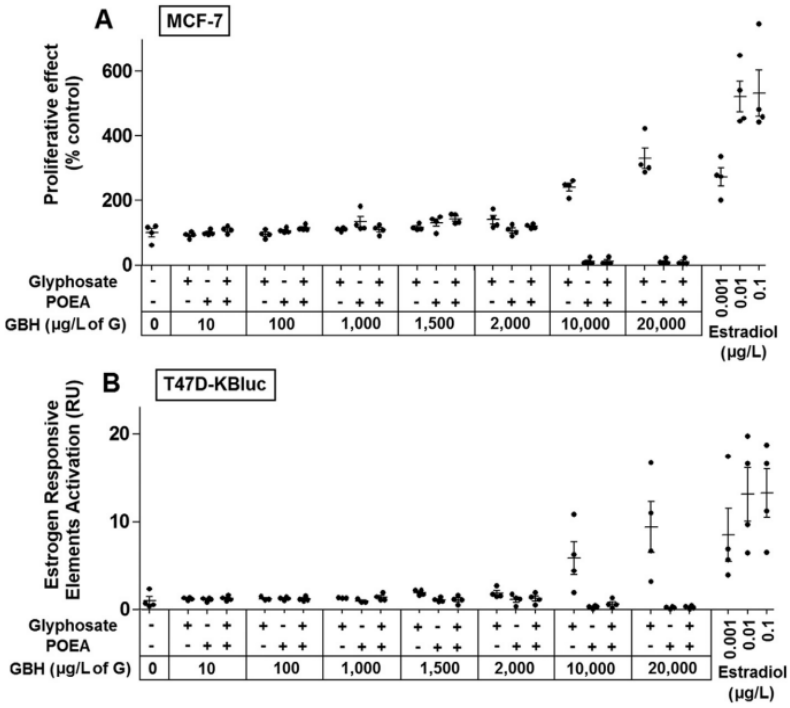


Fig. 2. Comparison of cell growth and estrogen receptor activation in human breast cancer cells treated with glyphosate, a GBH (Glyphogan), or POEA: a glyphosate-based herbicide adjuvant (polyethoxylated tallow amine; POEA) is not estrogenic in human mammary cells. (**A**) Proliferative effect of glyphosate alone, a glyphosate-based herbicide containing the adjuvant POEA (Glyphogan), or POEA alone in the E-Screen bioassay. Hormone-dependent MCF-7 human breast cancer cells were cultured under hormone-free conditions (n = 4) and proliferative effects measured by MTT assay following 6 days exposure to either the natural hormone (estradiol) or test substance (Glyphogan, POEA). (**B**) Glyphosate but not Glyphogan Roundup formulation or POEA stimulate ERE-luciferase reporter gene expression in T47D-Kluc cells. T47D-Kluc cells were treated for 24 h with the test substances and then subjected to a bioluminescence luciferase reporter gene assay (n = 4). +/−, indicates the presence or absence of the indicated chemical.

ERE-luciferase reporter gene assay

Glyphosate stimulated ERE-mediated transcription of the luciferase reporter gene starting at a concentration of 1,000 µg/L. Roundup Probio did not show ER activity at a glyphosate equivalent concentration, which induced a cell proliferative effect when glyphosate is tested alone. This may be explained by the potentially higher toxicity of the glyphosate-based formulation, which could have resulted in cell death at the higher concentrations tested. Two other commercial formulations, Roundup Original DI and Roundup Grand Travaux Plus, also gave negative results. To evaluate whether the luciferase reporter gene stimulatory effects observed with glyphosate were mediated through the ER, experiments were performed with the addition of the potent ER antagonist ICI 182,780. This antagonist was effective at suppressing ER activation induced by 0.001 and 0.01 µg/L but not at 0.1 µg/L of 17β-estradiol. The addition of ICI 182,780 effectively blocked the stimulatory effects of glyphosate at 2,000-20,000 µg/L, confirming its agonist-like mode of action.

Microarray gene expression profiling

MCF-7 cells treated for 48 hours with glyphosate, Roundup Probio, POEA, or bisphenol A, and 17β-estradiol were subjected to full transcriptome profiling. The analysis of gene ontology confirms that genes having their expression altered by treatment of MCF-7 cells with 10,000 µg/L glyphosate were involved in cell cycle regulation, as well as in stimulation by steroid hormones. The transcriptome of glyphosate-treated cells was also reflective of cell death through apoptosis. Roundup ProBio was assessed at the glyphosate equivalent concentrations of 1 µg/L (environmental level), 100 µg/L and 1000 µg/L (showing a cell proliferative trend). The statistical analysis of differential expression showed that genes having their function altered by Roundup or POEA had low fold changes. No genes whose expression was increased or repressed by POEA showed fold changes higher than 2. The study of transcriptome profiles shows that POEA alone is unlikely to have estrogenic effects. This was confirmed in that none of the treatments exhibited statistically significant correlations to the ER biomarker

Table 2
List of the 10 most up- or down-regulated genes after the treatment by glyphosate, Roundup, POEA, bisphenol A, or 17β-estradiol. MCF-7 cells treated by glyphosate (1 µg/L, 100 µg/L, 10,000 µg/L), Roundup (1 µg/L of glyphosate, 100 µg/L of glyphosate, 1000 µg/L of glyphosate), by the adjuvant POEA, by bisphenol A (80 µg/L) or by the positive control estradiol were subjected to a full transcriptome microarray analysis.

17β-estradiol (0.0027 µg/L)		17β-estradiol (0.027 µg/L)		17β-estradiol (0.27 µg/L)		Bisphenol A (80 µg/L)		POEA (100 µg/L)		POEA (10 µg/L)	
ALDH1A3	0.4	ALDH1A3	0.2	ALDH1A3	0.2	SEMA5A	0.3	NRF1	0.6	RNU7-163P	0.6
SEMA5A	0.4	PRICKLE2-AS3	0.2	PSCA	0.2	ALDH1A3	0.3	RNU6-1281P	0.6	SNORA54	0.6
PPARG	0.5	SEMA5A	0.3	TFPI	0.2	PSCA	0.3	RP3-486I3.5	0.6	IREB2	0.6
PSCA	0.5	PSCA	0.3	PRICKLE2-AS3	0.2	PRICKLE2-AS3	0.3	SNORA54	0.7	LINC00403	0.6
IGFBPL1	0.5	EPAS1	0.3	EPAS1	0.3	GABBR2	0.3	RNA5SP144	0.7	RP11-10N23.2	0.6
EPAS1	0.5	TFPI	0.3	LIPH	0.3	EPAS1	0.3	RP3-425P12.5	0.7	ANKRD10-IT1	0.6
PSCA	0.5	LIPH	0.3	SEMA5A	0.3	LIPH	0.4	RNU7-75P	0.7	COX17	0.6
S1PR3	0.5	SAT1	0.3	DAB2	0.3	PPARG	0.4	RNU6-1024P	0.7	RP11-473O4.4	0.6
MIR3911	0.5	PSCA	0.3	SAT1	0.3	PSCA	0.4	HIST2H2AB	0.7	RNU6-385P	0.6
RP11-398B16.2	0.5	RND1	0.3	PSCA	0.3	UPK2	0.4	AC099344.2	0.7	ACTL6A	0.6
RNU6-807P	2.3	EGR3	5.2	MYBL1	5.5	MYB-AS1	2.8	RP5-865N13.1	1.4	LOC339978	1.4
MYB-AS1	2.5	MYBL1	5.5	EGR3	5.6	MGP	2.9	LOC100506142	1.4	LOC100134937	1.4
STC1	2.5	MYB	6.0	AC106875.1	6.4	AC005534.8	3.1	FKSG29	1.4	FLJ45079	1.4
AC005534.8	2.6	AC106875.1	6.3	GREB1	6.5	AC106875.1	3.3	RNA5SP39	1.4	MIR99A	1.4
MYB	3.0	GREB1	6.6	MYBL1	6.5	GREB1	3.3	TBX3	1.4	RP11-61L14.6	1.5
AC106875.1	3.2	GREB1	7.1	GREB1	7.1	MYB	3.7	LOC101927783	1.5	DHRS3	1.5
GREB1	3.2	MGP	7.2	PGR	7.1	GREB1	3.8	LINC00396	1.5	RP11-148B18.4	1.5
PGR	3.4	PGR	7.8	MGP	9.0	PGR	4.2	FLJ45513	1.5	MIR103B2	1.6
GREB1	3.7	PGR	10.0	PGR	9.2	MGP	4.2	MIR99A	1.5	MIR103A2	1.7
PGR	4.6	MGP	12.4	MGP	15.0	PGR	6.2	RNU6-601P	1.6	MIR425	1.8
Glyphosate (1 µg/L)		**Glyphosate (100 µg/L)**		**Glyphosate (10,000 µg/L)**		**Roundup (1000 µg/L of G)**		**Roundup (100 µg/L of G)**		**Roundup (1 µg/L of G)**	
RNU6-385P	0.5	RP3-486I3.5	0.4	SEMA5A	0.5	SNORA54	0.5	MIR548A2	0.5	LRCH3	0.6
TNXB	0.6	RNU7-141P	0.5	DXO	0.5	RNU4ATAC	0.5	NFE2L3	0.5	MIR4328	0.6
RNA5SP303	0.6	MYB	0.6	ALDH1A3	0.6	TNXB	0.6	RNU6-177P	0.5	LOC101928000	0.6
RNU6-667P	0.6	RNU7-172P	0.6	PRICKLE2-AS3	0.6	RNU6-611P	0.7	PGR	0.5	RNU5B-4P	0.7
RP11-276G3.1	0.7	RNU7-113P	0.6	RNU7-134P	0.6	TICAM2	0.7	SNORD58A	0.6	ZNF404	0.7
RNU6-611P	0.7	RNA5SP303	0.6	XXbac-BPG254F23.7	0.6	AC005304.1	0.7	RNU6-998P	0.6	RNU6-322P	0.7
TGFB1	0.7	MIR3662	0.6	RP11-417B4.2	0.6	RNU6-893P	0.7	LINC00403	0.6	ADAM20P1	0.7
CHIC1	0.7	RNU6-1024P	0.6	AC073046.25	0.6	ZNF202	0.7	TMEM123	0.6	RP11-77M5.1	0.7
CCIN	0.7	SNORD20	0.6	AAK1	0.6	VPS35	0.7	LOC101928000	0.6	RAD54L2	0.7
RP11-473E2.2	0.7	RNU6-998P	0.7	LIPH	0.6	MIR4514	0.7	MIR3140	0.6	RP11-276G3.1	0.7
CRTAP	1.5	PDE11A	1.5	AC005534.8	1.6	CRTAP	1.4	AL592528.1	1.5	AP001469.5	1.4
PTPRS	1.5	MIR3074	1.6	SNORA70B	1.6	MAST4-AS1	1.4	RNU6ATAC18P	1.5	PLEKHA4	1.4
SNORA5A	1.5	AC010894.3	1.6	MYB-AS1	1.7	SLC25A30-AS1	1.4	PAIP2B	1.5	SNORA70B	1.5
RP11-575A19.2	1.5	RNU6-306P	1.7	STC1	1.8	RNU6-853P	1.4	RP11-319F12.2	1.5	PCAT1	1.5
RNU6-1258P	1.5	RNU4-38P	1.7	MIR99A	1.8	RNU4-40P	1.5	SART3	1.6	ATP11A-AS1	1.5
RN7SKP56	1.5	MIR99A	1.7	AC106875.1	2.0	SNORA5A	1.5	RNA5SP498	1.6	RP5-865N13.1	1.5
LOC729451	1.5	MIR532	1.8	GREB1	2.1	RP5-1022J11.2	1.5	RNA5SP498	1.6	MIR4733	1.5
MIR532	1.6	MIR501	1.8	MYB	2.2	RP11-218L14.4	1.5	RP11-300E4.2	1.6	LOC101927557	1.5
ALG9	1.6	RNU6-1004P	2.3	PGR	2.3	RNU4-38P	2.0	RNU6-388P	1.7	SNORD41	1.7
SNORD59B	1.6	RNU4-60P	3.1	GREB1	2.4	SNORD1A	2.2	MIR4256	2.3	RNU4-62P	1.9

RNA-sequencing gene expression profiling

In order to confirm endocrine disturbances provoked by glyphosate, RNA extracted from MCF-7 cells treated with glyphosate (10,000 µg/L), estradiol (0.27 µg /L) or bisphenol A (80 µg/L) for 48 hours were subjected to a full RNA-Seq analysis using the Illumina sequencing platform. Although their gene expression profile were different, MCF-7 cells treated by these 3 chemicals presented alterations reflecting a response to steroid hormones and a modulation of cell proliferation, although the significance of the overlapping genes and those in these pathways was lower for glyphosate than the other compounds. Overall, the RNA-seq method was more sensitive and identified 2-3 times more genes whose expression was significantly altered compared to the microarray approach. A total of 5102, 2939 and 1083 genes had their expression significantly disturbed by estradiol, BPA and glyphosate, respectively. Afterwards the ER gene expression biomarker was applied to see if ERα agonist effects could be detected after transcriptome profiling using RNA-Seq platforms. The results were similar to those obtained using the microarray data. Glyphosate failed to pass the threshold of significance for ERα activation. Although the RNA-seq platform was able to identify more statistically significant genes than microarrays, the genes altered differed between the two methods. Among those that are part of the ERa activation biomarker, the genes that were altered by glyphosate in the microarray analysis (CD44, PGR, and MYB) were different from those identified by RNA-seq (CXCl12, ABHD2, and EFNA1).

Molecular dynamics simulation and ONIOM binding energy calculations

The ability of glyphosate to bind to ERα was evaluated by molecular dynamics simulations and ONIOM calculations. Results from the molecular dynamics simulations of glyphosate-ER interactions reveal that glyphosate enters the active site with a large number of water molecules. The glyphosate phosphonate group interacts with ARG 394 by creating hydrogen bonds. It was noted that glyphosate is unlikely to interact with HIS 524, a residue having a pivotal role in maintaining protein structure in the biologically active agonist conformation. The results of the ONIOM binding energy assessment strongly imply that the binding of glyphosate at the active site of the receptor is weak and unstable, suggesting that glyphosate is unlikely to bind to ERα.

Discussion

The results of this study suggest that glyphosate is a weak activator of ERα in hormone-dependent human breast cancer cells. The glyphosate intake necessary to reach a systemic concentration representative of the estrogenic effects shown in this study would only be encountered in cases of extreme exposures (incidental ingestion, mishandling). An evaluation of the glyphosate binding energy confirmed that this compound is unlikely to activate the ER. The presence of glyphosate-associated cytotoxic effects could explain the discrepancies between the results we obtained with the ERα biomarker and those from the cellular assays. It is thus plausible that glyphosate is activating ERα through a ligand-independent mechanism albeit at high concentrations. To determine any estrogenic potential of adjuvant co-formulants, a number of glyphosate-based formulations were tested and no estrogenic effects could be demonstrated. However, cytotoxicity was observed at glyphosate-equivalent concentrations lower than those required to elicit a proliferative response to glyphosate alone.

Study conclusion

This study has demonstrated that glyphosate activates ERα in breast cancer cells but only at relatively high concentrations, and that this activation happens through a ligand-independent pathway. The authors concluded that the results suggest that humans exposed to glyphosate would not exhibit ER activation at typical exposure levels.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The objective of this study was to evaluate the possible estrogenicity of glyphosate and glyphosate-based formulations and their adjuvants. The tests performed were the E-screen using different cell lines, the ERE-luciferase reporter gene assay, mmicroarray gene expression profiling and RNA-sequencing gene expression profiling. An increase in cell proliferation was observed in human breast cancer cells (MCF-7) at 10,000 µg/L and reached a maximum response at 1,000,000 µg/L. Similar but less pronounced results were observed with the T47D cell line. Glyphosate stimulated ERE-mediated transcription of the luciferase reporter gene starting at a concentration of 1,000 µg/L. The analysis of gene ontology confirms that genes having their expression altered by treatment of MCF-7 cells with glyphosate were involved in cell cycle regulation, stimulation by steroid hormones and cell death through apoptosis. ONIOM binding energy assessment strongly implies that the binding of glyphosate at the active site of the estrogen receptor is weak and unstable, suggesting that glyphosate is

unlikely to bind to ERα.

This study has demonstrated that glyphosate activates ERα through a ligand-independent pathway only at high concentrations that are not encountered at typical exposure levels. This publication is considered relevant for glyphosate risk assessment and reliable without restrictions.

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

Publication: Mesnage *et al*., 2017	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 98.0 %. Source: Sigma-Aldrich (UK).
Only glyphosate acid or one of its salts is the tested substance	Y	Also glyphosate based formulations and surfactants were tested.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
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	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This study has demonstrated that glyphosate activates ERα through a ligand-independent pathway only at high concentrations that are not encountered at typical exposure levels. This publication is considered relevant for glyphosate risk assessment and reliable without restrictions.		

Assessment and conclusion by RMS:

The study is concluded to be reliable (Klimisch Score 1).

While the study does show that glyphosate activates the ERα, the effects only occurred at high dose concentrations which are not realistic for *in vivo* situations.

B.6.8.3.14. Public literature – Effect on ER-mediated transcriptional activity

Data point:	CA 5.8.3
Report author	Thongprakaisang, S. *et al.*

Report year	2013
Report title	Glyphosate induces human breast cancer cells growth via estrogen receptors
Document No	doi.org/10.1016/j fct.2013.05.057 E-ISSN: 1873-6351. L-ISSN: 0278-6915
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable with restrictions

Executive Summary
This study focuses on the effects of pure glyphosate on estrogen receptors (ERs) mediated transcriptional activity and their expressions. Glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, but not in hormone-independent breast cancer, MDA-MB231 cells, at 10^{-12} to 10^{-6} M in estrogen withdrawal condition. The proliferative concentrations of glyphosate that induced the activation of estrogen response element (ERE) transcription activity were 5-13 fold of control in T47D-KBluc cells and this activation was inhibited by an estrogen antagonist, ICI 182780, indicating that the estrogenic activity of glyphosate was mediated via ERs. Furthermore, glyphosate also altered both ERα and β expression. These results indicated that low and environmentally relevant concentrations of glyphosate possessed estrogenic activity.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material
Glyphosate (purity >98%) was obtained from AccuStandard (New Haven, CT, USA).

Cell lines and culture conditions
A hormone-dependent human breast cancer cell line (T47D), a stably ERE-luc construct transfected hormone-dependent breast cancer cell line (T47D-KBluc) and a hormone-independent human breast cancer cell line (MDA-MB231) were obtained from the American Type Culture Collection (ATCC). Cells were maintained in recommended standard medium.

B. STUDY DESIGN

In vitro estrogen receptor activation-reporter assay
To study the estrogenicity and/or anti-estrogenicity of glyphosate, the T47D-KBluc cell line, stably transfected with a triplet ERE (estrogen response element)-promoter-luciferase reporter gene construct was used in this study. To minimize the effect of estrogen in the medium, 5 days prior to the start of the assay, cells were incubated in a non-phenol red RPMI modified medium with replacement of 10% FBS by 10% dextran-charcoal treated FBS (CSS). One day prior to the assay, cells were seeded at 10^4 cells/100 µL/well and were allowed to attach overnight. The dosing medium was further modified by reduction to 5 % CSS and then replaced with 100 µL/well of dosing medium containing glyphosate at concentrations ranging from 10^{-12} to 10^{-6} M. Estradiol (E2) in the same range of concentrations was used as the positive control. Dosing medium without glyphosate was used as the negative control and wells without cells were used as the blank. After 24 hours of incubation, cells were washed with 100 µL PBS and harvested in 25 µL lysis buffer. The luciferase assay was performed by injecting 50 µL of reaction buffer and 50 µL of 1 mM D-luciferin and fluorescent intensity was measured by means of the microplate luminometer. Luciferase activity was quantified as relative light units (RLU).

Cell viability MTT assay
Cell growth and cell viability were tested using the 3-(4,5-dimetylthiazol, 2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent assay. Cells were seeded at 10^4 cells/100 µL/well in 96-well microtiter plates. For the E2

withdrawal condition, cells were cultured in 10% CSS and a non-phenol red RPMI medium for 4 days before seeding. After 24 hours the cells were treated with E2 or glyphosate at concentrations ranging from 10^{-12} to 10^{-6} M. For E2 receptor antagonist conditions, E2- or glyphosate-treated cells were co-incubated with ICI 182780 at 1 and 10 nM. Cell sensitivity to a chemical was expressed as the % cell viability compared to control cells.

Western blot analysis
Whole-cell extracts were prepared from cells treated for 6 and 24 hours with 10^{-12}, 10^{-9}, and 10^{-7} M glyphosate and non-treated control. The cells were lysed, incubated on ice and centrifuged. The supernatants were collected and either processed or stored at -80 °C until use. The protein concentration was measured using Bradford reagent and each lysate was aliquot for an equal amount of protein, 30 µg, before mixing with Laemmli loading buffer and then boiled at 95 °C for 5 minutes. The samples were resolved over 7.5 % polyacrylamide-SDS gels and transferred to a nitrocellulose membrane using a Mini Trans-Blot Electrophoretic Transfer Cell. The membrane was treated with blocking solution for one hour at room temperature and subsequently probed overnight with primary antibody (ERα, ERβ or β-actin) and then rinsed. HRP-conjugated secondary antibodies were added to the membrane for 2 hours and the membranes rinsed with TBS-T. Protein visualization was achieved by using enhanced chemiluminescence and the emitted light was captured on film. The signals on the films were quantified using densitometry.

Cell number counting
T47D Cells were prepared in E2 withdrawal conditions 4 days before the start of the assay. Cells were placed in a 24 well culture plate with 10^4 cells/mL/well and incubated overnight. The medium was then replaced with 1 mL of treatment solution and incubated for 72 hours. Afterwards, the cells were washed with 1 mL PBS and then 100 µL of a trypsin–EDTA solution was added to detach. An aliquot of the cells was taken for counting using a counter analyzer.

Statistical analysis
Data are presented as the means ± SE. Statistical significance was determined using the Student's t-test. A two-tailed $p<0.05$ was evaluated as a statistically significant difference.

II. RESULTS AND DISCUSSION

T47D, hormone-dependent breast cancer cell growth
The hormone-dependent T47D and hormone-independent MDA-MB231 cell lines were studied both in completed medium and estrogen withdrawal medium to differentiate the effect of glyphosate from that of endogenous estrogen. Estrogen in a concentration range of 10^{-12} to 10^{-6} M was used as the positive control. Cell growth was assessed using the MTT cell viability assay. The results showed that T47D and MDA-MB231 cells exhibited different patterns of responses to glyphosate. In the absence of E2, glyphosate produced cell proliferation in T47D cells of approximately 15–30%. This effect was about half of the E2 response which is the most potent agonist in hormone dependent ER-positive breast cancer cells. No effect was observed on cell proliferation in MDA-MB231 cells both in the absence or presence of E2.

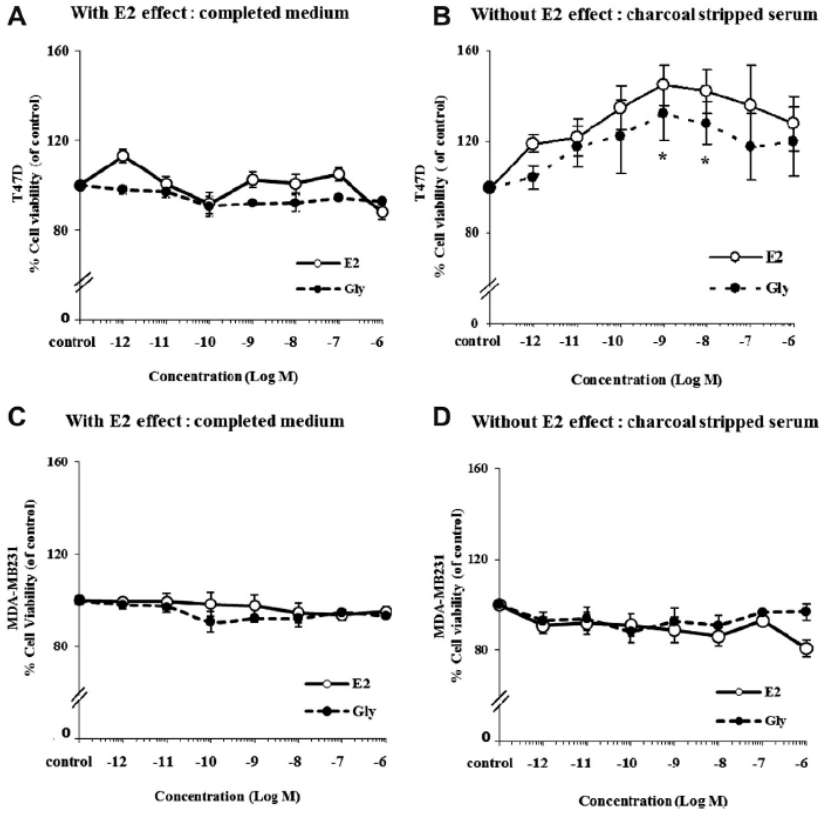


Fig. 1. Concentration-effect relationship of E2 and glyphosate on human breast cancer T47D (A and B) and MDA-MB231 cells (C and D). Cells were treated with varying concentrations ranging from 10^{-12} to 10^{-6} M of E2 and glyphosate. Cell viability was compared between in completed medium (A and C) and in hormone withdrawal medium (B and D) by using MTT assay at 24 h ($n = 3$, $^{*} = p \leqslant 0.05$, significantly different as compared to control).

Cell proliferation via estrogen receptors

Due to the fact that the proliferative effect of glyphosate occurred only in T47D cells in the absence of E2, it was hypothesized that ER signaling may be involved in glyphosate-induced cell proliferation. Therefore, the effect of glyphosate on T47D cells was investigated in the presence of an ER antagonist, ICI 182780, to inhibit the estrogen receptor mediated response. The effective concentration of 1 nM of ICI 182780 was added to varying concentrations of glyphosate and E2 to observe its antagonistic activity. The results showed that ICI 182780 at 1 nM mitigated the proliferative effects of both glyphosate and E2. A higher concentration of ICI 182780 (10 nM) completely inhibited the growth promoting effects of glyphosate. These results suggest that glyphosate may produce the proliferative effect via the ER.

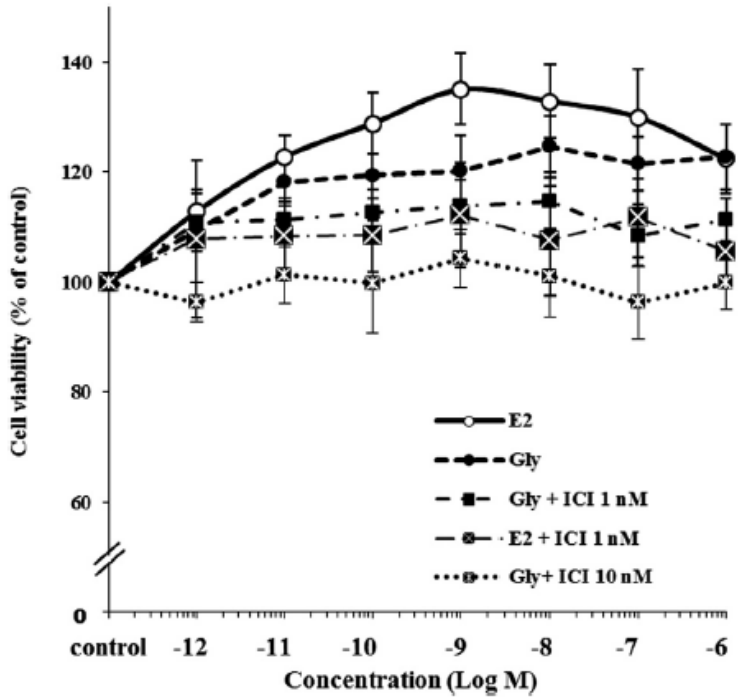


Fig. 2. Proliferative effects of E2 and glyphosate on human breast cancer T47D cells. T47D cells were treated with varying concentrations of E2 and glyphosate ranging from 10^{-12} to 10^{-6} M and co-incubation with ICI 182780 (1 or 10 nM). Cells were cultured in hormone withdrawal medium for 5 days prior treatments. The cell viability was detected by MTT assay at 24 h. Each point was plotted from the mean value of three independent experiments ± SE as shown in the graph ($^{*}p \leqslant 0.05$ significantly different as compared to glyphosate alone).

ERE-transcription activity via estrogen receptors

T47D-KBluc cells, stably transfected with a triplet ERE (estrogen response element)-promoter-luciferase reporter gene construct, were treated with the concentrations of glyphosate that produced cell proliferation. The results showed that glyphosate in a concentration range of 10^{-12} to 10^{-6} M induced ERE activation 5- to 13-fold of control and these effects were less than about half of that induced by E2. Glyphosate co-incubation with the ER antagonist, ICI 182780, exhibited a significant reduction in responses. ICI 182780 at 10 nM completely inhibited the ERE transcriptional activity of glyphosate. Since glyphosate was shown to induce cell proliferation and ERE activation via the ER, the potential effects of glyphosate on endogenous E2 signaling was investigated. Cells were co-incubated with glyphosate and E2 and the results revealed that glyphosate suppressed E2-induced ERE activation suggesting that glyphosate behaves as an ER antagonist in the presence of E2.

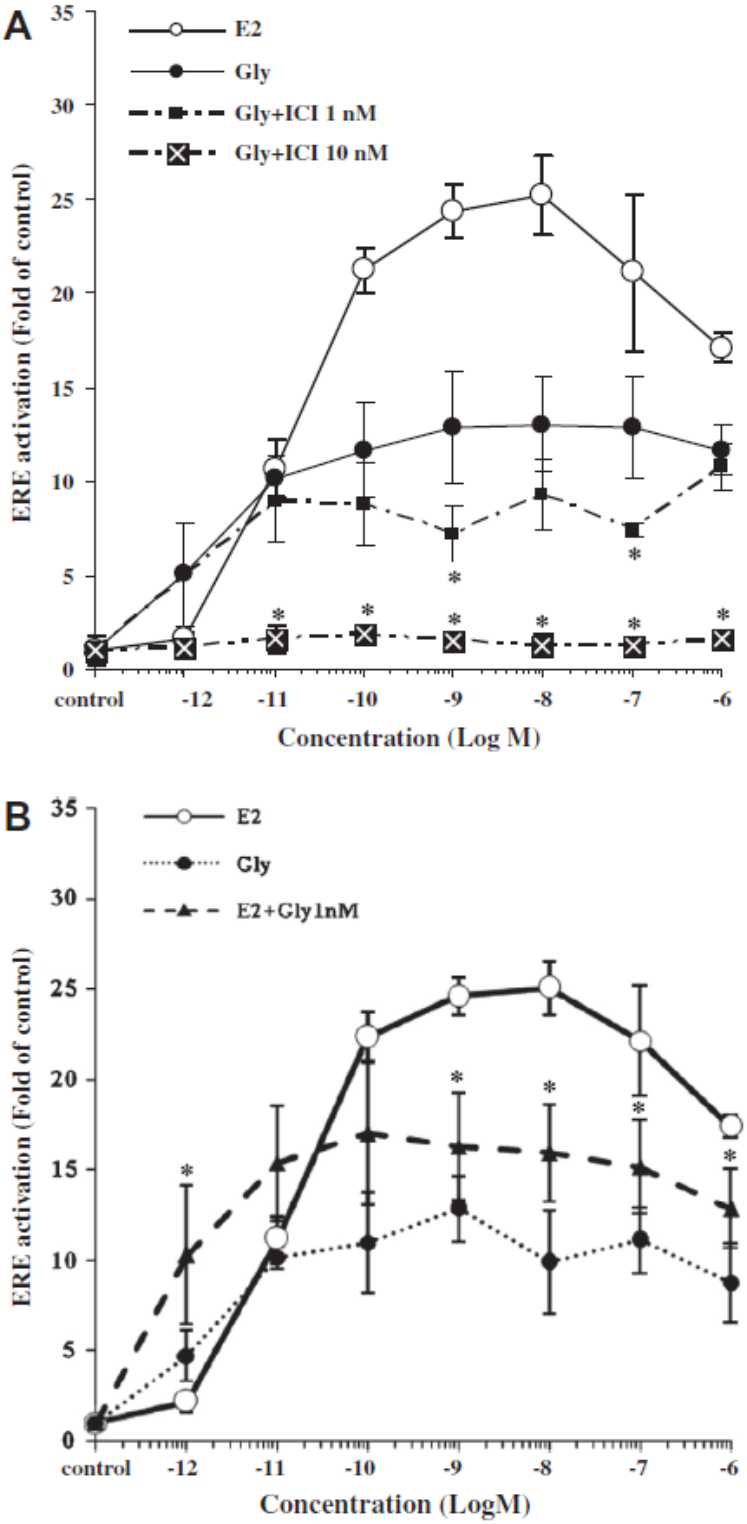


Fig. 3. The effects of 17β-estradiol (E2), glyphosate, and glyphosate co-incubation with ICI 182780 on ERE transcription activity in T47D-KBluc cells (A). Cells were cultured in E2 withdrawal medium for 5 days before the treatment in each experiment. ICI 182780 at the concentrations 1 and 10 nM were used. The experiment was observed at 24 h treatment ($n = 3$, $^{*} = p \leqslant 0.05$, significantly different as compared to glyphosate alone). Glyphosate at 1 nM suppressed to the E2 effects along varying concentrations (B) ($n = 4$, $^{*}p \leqslant 0.05$ significantly different as compared to glyphosate alone).

Expression of ERα and ERβ in human breast cancer cells

The expression of proteins involved in ERs including ERα and ERβ, was studied with the Western blot technique. After 6 hours of exposure, glyphosate increased the levels of both ERα and ERβ in a concentration-dependent manner while after 24 hours only ERα showed a significant induction at the highest glyphosate concentration tested (10^{-7} M). This result suggests that glyphosate alters the expression of both ERα and ERβ in human breast cancer cells.

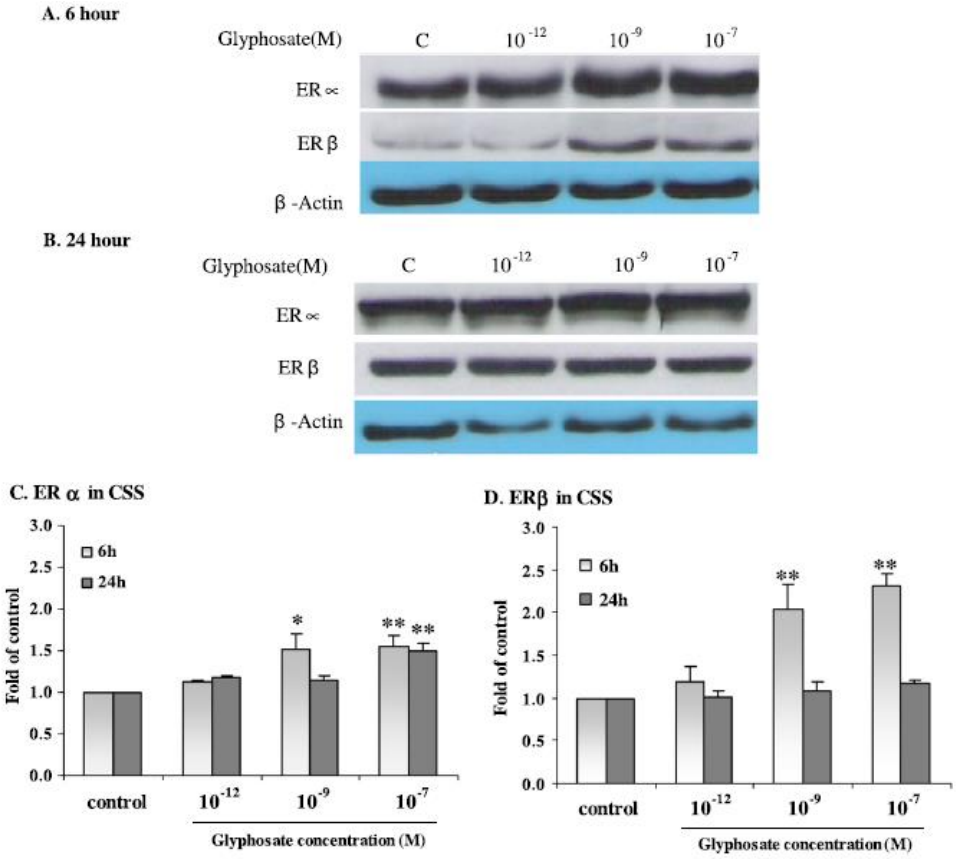


Fig. 4. The effects of glyphosate on ERα and β expression. E2-withdrawal T47D cells were used. (A) 6 h and (B) 24 h incubation time showed specific band of ERα, ERβ (66 kDa) and β-actin (44 kDa), a representative sample from one experiment. Optical densities of specific band ERα and ERβ were determined from western blot and each band was normalized to the β-actin band. The normalized mean of three replications ± SE optical density values are shown in the histogram of ERα (C) and ERβ (D) with * = $p \leq 0.05$, ** = $p \leq 0.01$ significantly different as compared to control.

Discussion

In this study, glyphosate was found to increase cell proliferation of a hormone dependent breast cancer T47D cell line at concentrations ranging from 10^{-12} to 10^{-6} M while this effect was not observed in a hormone independent breast cancer MDA-MB231 cell line. The results from the ERE luciferase assay confirmed ER activation because the responses seen could be blocked by the ER antagonist ICI 182780. Glyphosate induced rapid activation of ERβ while activation of ERα was slower but prolonged. It is suggested that glyphosate may behave like a weak xenoestrogen which can activate both subtypes of ER but with a different time course. Although the nature of the binding of glyphosate to the ER is still unknown, the ability of glyphosate to stimulate the ERE-gene transcription activity and upregulation of ERα protein expression suggests that glyphosate may exert the stimulatory effects via an ER-dependent mechanism.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The objective of this study was to investigate the possible estrogenic effect of glyphosate and its mode of action. The endpoints explored were the cell proliferation of hormone-dependent and hormone-independent cell lines with and without an ER antagonist, ERE-transcription activity with and without an ER antagonist, and

expression of ERs. Glyphosate was found to produce cell proliferation in a hormone-dependent cancer cell line but not in a hormone-independent cancer cell line in the absence of E2. In the presence of a potent ER antagonist the cell proliferation caused by glyphosate in a hormone-dependent cancer cell line was reduced. The interaction of glyphosate with the ER was confirmed by ERE activation with and without an ER antagonist. When cells were co-incubated with glyphosate and E2, glyphosate suppressed the E2-induced ERE activation suggesting that glyphosate behaves as an antagonist in the presence of an endogenous agonist. It was demonstrated that glyphosate alters the expression of both ERα and ERβ in human breast cancer cells.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test results are not corroborated by *in vivo* regulatory ED toxicology studies such as the uterotrophic assay and the female pubertal assay (U.S. EPA Endocrine Disruptor Screening Program).

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

Publication: Thongprakaisang *et al*., 2013	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of >98 %. Source: AccuStandard, New Haven, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
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	Y	The slight estrogenic effect of glyphosate reported was not confirmed in *in vivo* studies such as the uterotrophic assay and the female pubertal assay.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	Results not consistent with other publications on ED.
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test results are not corroborated by *in vivo* regulatory ED toxicology studies such as the uterotrophic assay and the female pubertal assay.		

Assessment and conclusion by RMS:

The study by Thongprakaisang, S. *et al.* 2013 was referred to by Mesnage, R. *et al.* 2017 which is summarised in section B.6.8.3.13. They noted the striking difference in concentrations of glyphosate where effects are observed

between the two studies. In the study by Thongprakaisang effects start at concentrations that are comparable to those of estradiol although the magnitude of the effect is somewhat lower. In contrast in the study by Mesnage et al. 2017 effects were only observed at very high *in vitro* concentrations. Mesnage *et al.* 2017 could not attribute this to a difference in the study design and stated that „*the finding of Thongprakaisang and colleagues that a dose of 10^{-12} M of glyphosate had a greater estrogenic effect than estradiol raises major concerns and suggests the possible presence of contaminants".*

The fact that effects were observed in the study by Thongprakaisang et al. 2013 at concentrations as low as 10^{-12} M is indeed surprising and does not appear to be in line with the other glyphosate studies. Therefore, the study is concluded to be reliable with restrictions.

B.6.8.3.15. Public literature – Effect on ERα- and ERβ

Data point:	CA 5.8.3/016
Report author	Brennan, J.C. *et al.*
Report year	2016
Report title	Development of a recombinant human ovarian (BG1) cell line containing estrogen receptor alpha and beta for improved detection of estrogenic/antiestrogenic chemicals. and its corrigendum
Reference	Environmental Toxicology and Chemistry, 2016, Vol. 35 (1): pp. 91-100
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable with restrictions

Data point:	CA 5.8.3/017
Report author	Brennan, J.C. *et al.*
Report year	2017
Report title	Supplemental information to: Development of a recombinant human ovarian (BG1) cell line containing estrogen receptor alpha and beta for improved detection of estrogenic/antiestrogenic chemicals.
Reference	Environmental Toxicology and Chemistry, 2017, Vol. 36 (5): p. 1405
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Not applicable

Executive Summary

The study was based on an estrogen-responsive recombinant human breast cancer cell line (VM7Luc4E2) that was accepted by the US Environmental Protection Agency (USEPA) and Organisation for Economic Co-operation and Development (OECD) as a bioanalytical method to detect estrogen receptor (ER)

agonists/antagonists. This cell line contains only 1 of the 2 known ER isoforms, ERα but not ERβ, and the differential ligand selectivity of these ERs indicates that the currently accepted screening method only detects a subset of total estrogenic chemicals. To improve the estrogen screening bioassay, VM7Luc4E2 cells, which are derived from a human breast cancer cell line (MCF7 cells), were stably transfected with an ERβ expression plasmid and positive clones identified using ERβ-selective ligands. A highly responsive clone (VM7LucERβc9) was identified that exhibited greater sensitivity and responsiveness to ERβ-selective ligands than VM7Luc4E2 cells, and quantitative reverse-transcription polymerase chain reaction confirmed the presence of ERβ expression in these cells. Screening of pesticides and industrial chemicals identified chemicals that preferentially stimulated ERβ-dependent reporter gene expression. Together, these results demonstrate the utility of this dual-ER recombinant cell line for detecting a broader range of estrogenic chemicals than the current VM7Luc4E2 cell line. Additionally, screening with both cell lines allows identification of ERα- and ERβ-selective chemicals.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material
Glyphosate (no data on purity or CAS number) (vehicle control: Dimethyl sulphoxide (DMSO, no data on purity); positive control: 17β-Estradiol (E2; no data on purity))

Cell lines and culture conditions
VM7Luc4E2 and VM7LucERβc9 cell lines were used in the studies with glyphosate. The VM7Luc4E2 cell line was developed by the authors and is used and accepted by USEPA and OECD for determination of ER agonists/antagonists. The VM7LucERβc9 was produced by stable transfection of the VM7LucERβc9 cells with ERβ/pcDNA3.1+Zeo. For routine maintenance, cells were grown in α-minimal essential medium containing 10% fetal bovine serum and additional 600 mg/L Zeocin for the VM7LucERβc9 cells.

B. STUDY DESIGN

Stable transfection
To produce the stable VM7LucERβ cell line, human ovarian carcinoma (VM7Luc4E2) cells containing a stably transfected estrogen-responsive luciferase plasmid were transfected with ERβ/pcDNA3.1+Zeo using FuGene6 transfection reagent according to the manufacturer's recommendation. After 24 h incubation in regular medium, the transfected cells were split 1 to 15 and replated into selective medium containing the antibiotic Zeocin (600 mg/L) which was replaced every 3 d with fresh Zeocin-containing medium. After approximately 3 weeks of growth, 28 individual cell colonies were isolated, their ERβ responsiveness was determined, and those clones exhibiting the greatest induction by ERβ-selective ligands (Br-ERβ-04 [31.6 nM] and genistein [10 nM]), were selected for further evaluation.

RNA isolation and RT-PCR
Cells in 10 cm plates (4 replicates per cell line) were grown in maintenance medium with the addition of Zeocin for the VM7LucERβc9 cells. Total RNA was isolated using RNeasy (Qiagen) according to the manufacturer's instructions (β-mercaptoethanol was added to the lysis buffer immediately before use, and cells were homogenized using QIAshredder homogenizer). Complementary DNA (cDNA) was generated from 2 mg RNA using a high-capacity cDNA Reverse Transcription kit (Applied Biosystems) with random primers followed by a 1:10 dilution with RNAse/DNAse-free water. Real-time quantitative polymerase chain reactions (20 mL) were performed with TaqMan Fast Universal PCR Master Mix and TaqMan Gene Expression assays (Applied Biosystems). The ERα and ERβ messenger RNA (mRNA) levels were quantitated, normalized to β-actin (internal control), and results presented relative to levels in VM7Luc4E2 cells (set to value of 1.0). Primers or probes for human ERβ (ESR2, Hs01100353_m1), human ERα (ESR1, Hs00174860_m1), and human b-actin (ACTB, Hs999999031_m1) were obtained from Applied Biosystems.

Incubation and luciferase analysis

VM7LucERβ cells were switched from maintenance medium to estrogen-stripped medium, containing 10% charcoal-stripped fetal bovine serum and incubated 3 d before plating into white, clear-bottomed 96-well tissue culture plates at a density of 750 000 cells/mL. Cells were allowed to attach for 24 h and then were incubated with carrier solvent DMSO (1% final solvent concentration) or 10 µM glyphosate for 24 h at 37°C with triplicate wells per chemical and controls. After incubation, cells were rinsed twice with phosphate-buffered saline, lysed with Promega cell lysis buffer, and shaken for 20 min at room temperature to allow complete cell lysis. Luciferase activity in each well was measured by a microplate luminometer. For comparative purposes, luciferase induction values were normalized to maximal luciferase induction obtained with 1 nM E2 in each plate (set at 100%). Values represented the mean ± standard deviation (SD) of triplicate incubations in the single screening analysis of the chemical compound.

Statistical analysis

Significant differences between results were determined using one-way analysis of variance (i.e., Student's t test, 2-tailed, type 2, $p<0.05$). Luciferase activity of control (solvent-treated) cells was substracted from that of treated cells to obtain final induced activity (relative light units [RLU]). Final RLU values less than 0 were set at 0 RLUs. Half-maximal concentrations induced (50% effective concentration [EC50]) or repressed (50% inhibitory concentration [IC50]) by chemical or extract were determined using SigmaPlot (Ver 12) by the concentration of chemical that induced exactly 50% of maximal E2-induced luciferase activity.

II. RESULTS AND DISCUSSION

Estrogenic effects of glyphosate

Glyphosate (reported in the supplemental information) had no estrogenic effect in both VM7LucERβ and VM7LucERβ cells. The induction of luciferase activity was expressed as the mean ± SD of three replicate analyses of one exposure experiments. Values are presented as a percent of maximum E2 induction (set at 100%), and the mean result for glyphosate had been in the range of the solvent control. In the table below the solvent control, DMSO was set to 0% and E2 was set to 100%:

Compound	Activity [%] VM7Luc4E2	Activity [%] VM7LucERβc9
DMSO	0	0
Glyphosate	6 ± 2	2 ± 4
E2	100	100

III. CONCLUSIONS

Assessment and conclusion by applicant:

Glyphosate did not show any estrogenic activity at a concentration of 10 µM in two cell lines or via the two human estrogen receptor (hER) subtypes, hERα and hERβ. Based on the OECD 455 guideline for the VM7 assay, relative activity for the test substance that is < 10% of the response of a maximally inducing concentration of E2 is considered to be negative. In addition, when the VM7Luc4E2 cell line was validated[12] (previously known as BG1 cell line) the threshold for positive agonist activity was set at 20% of the response of a maximally inducing dose of E2. Therefore, glyphosate is concluded to have no ERα, ERβ agonistic activities, in vitro.

The estrogenic effect of glyphosate has been investigated in VM7LucERβ and VM7LucERβ cells. In the assay the induction of luciferase activity as a parameter for receptor activation was measured. The

[12] Interagency Coordinating Committee on the Validation of Alternative Methods. 2011. Independent Scientific Peer Review Panel Report: Evaluation of the LUMI-CELL® ER BG1Luc ER TA Test Method. Research Triangle Park, NC:National Institute of Environmental Health Sciences

induction of luciferase activity by glyphosate was found to be in the range of the solvent control.

The publication on in vitro estrogen receptor agonistic activity of pesticides indicates that, even though it does not follow a defined guideline, the study is well documented and is scientifically sound. Therefore, the study is reliable with restriction and is relevant for risk assessment.

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

Publication: Brennan, J.C. *et al.*, 2016	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Development a new cell line to improve the detection of estrogenic and antiestrogenic chemicals. The OECD TG 455 is partially respected.
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	
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	NA	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
Cytotoxicity tests reported	N	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	NA	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterized.		

Assessment and conclusion by RMS:

The study is concluded to be reliable with restrictions (Klimisch score 2). Limitations noted were no information on purity of the test material, unclear which concentrations were tested and only limited results provided for glyphosate (no individual data shown).

B.6.8.3.16. Public literature – Effect of co-formulants on Aromatase activity

Data point:	CA 5.8.3/018
Report author	Defarge, N. *et al.*
Report year	2016
Report title	Co-Formulants in Glyphosate-Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels
Document No	International Journal of Environmental Research and Public Health, 2016, Vol. 13 (264): pp. 1-17
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Study reliable (klimisch score 1)

Executive Summary
In this study endocrine disruption of co-formulants in six glyphosate-based herbicides were tested. All co-formulants and formulations were comparably cytotoxic well below the agricultural dilution of 1% (18–2000 times for co-formulants, 8–141 times for formulations), and not the declared active ingredient glyphosate alone. The endocrine-disrupting effects of all these compounds were measured on aromatase activity below the toxicity threshold. Aromatase activity was decreased both by the co-formulants alone (polyethoxylated tallow amine—POEA and alkyl polyglucoside—APG) and by the formulations, from concentrations 800 times lower than the agricultural dilutions; while glyphosate exerted an effect only at 1/3 of the agricultural dilution. It was demonstrated that endocrine disruption by glyphosate-based herbicides could not only be based on the active ingredient but also to co-formulants. These results could explain numerous *in vivo* results with glyphosate-based herbicides that were not seen with glyphosate alone.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material
This study tested formulations, co-formulants and glyphosate alone. The focus of this assessment is on the glyphosate alone data. Glyphosate (isopropylamine salt of n-phosphonomethylglycine) (no data on purity, CAS number 1071-83-6) (vehicle control: not further specified, positive control: Formestan (4-hydroxyandrost-4-ene-3,17-dione; no data on purity))

Cell lines and culture conditions
JEG3 cells (human placental choriocarcinoma cells) used in these assays are well-characterized and validated as useful models to test toxicities of pesticides, corresponding to what is observed in fresh tissue or primary cells.
JEG3 cells were grown in phenol red-free Eagle's minimum essential medium (EMEM) containing 2 mM glutamine, 1% non-essential amino acid, 100 U/mL of antibiotics (a mixture of penicillin, streptomycin and fungizone), 10 mg/mL of liquid kanamycin and 10% fetal bovine serum. JEG3 cells were supplemented with 1 mM sodium pyruvate. Cells were grown with this medium at 37°C (5% CO2, 95% air) during 48 h to 80% confluence, then washed and exposed 24 h with serum-free EMEM to glyphosate, glyphosate-based herbicide formulations and their co-formulants. Before treatment, all the glyphosate, glyphosate-based herbicide and co-formulants were diluted in serum-free medium and adjusted to a similar pH.

B. STUDY DESIGN

Cell treatment and cytotoxicity biomarkers
Confluent cells (80% of confluence) were washed with serum-free EMEM and then exposed to various

concentrations of glyphosate, glyphosate-based herbicide and co-formulants in EMEM serum-free medium for 24 h. After treatments, cytotoxicity was determined by an MTT assay (optical density measured at 570 nm). The bioluminescent Toxilight bioassay (Lonza, Saint Beauzire, France) was applied for the membrane degradation assessment by intracellular adenylate kinase (AK) release in the medium that is described as a necrosis marker. Glyphosate was tested from 1 to 10000 mg/L.

Determination of aromatase activity

Aromatase activity was evaluated according to the tritiated water release assay. This method is based on the stereo-specific release of 1β-hydrogen from the androstenedione substrate, which forms tritiated water during aromatization.

JEG3 cells were exposed for 22 h at 37°C (5% CO2, 95% air) to 700 µL of non-toxic doses of different xenobiotics. Formestane, a well-known aromatase inhibitor, was used as a positive control. Then 50 µL of 200 nM [1β-3H] androstenedione was added, and incubation went on for 120 min more. The reaction was stopped by placing the plates at 4°C for 10 min. Cell fragments were removed by 5 min centrifugation at 2000 rpm at 4°C and by addition of 1 mL of chloroform to the 500 µL supernatant. After 5 min centrifugation at 4000 rpm at 4°C, 0.5 mL of charcoal/dextran (0.25%/0.025%) was added. The mixture was gently agitated, rested at 4°C for 10 min, and centrifuged at 4000 rpm for 10 min at 4°C. Supernatant fractions (500 µL) were harvested in 6 mL vials and 4 mL Ultima Gold LLT was added. The mixture was assessed for radioactivity by a double 5 min scintillation counting. Glyphosate was tested at non-toxic doses in the aromatase assay (16 mg/L).

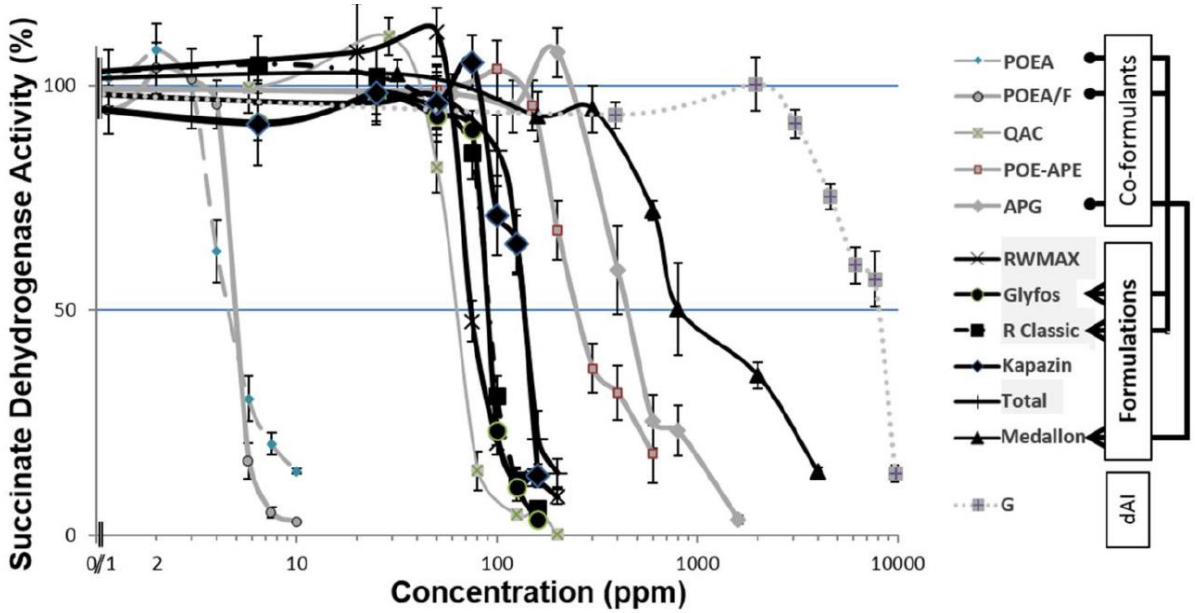
Statistical analysis

All results are means ± SEM. Three independent experiments were performed using triplicate cultures each. In MTT assays, LC50 values were the best-fitted value of a non-linear regression using asymmetric (5-parameters) equation with GraphPad Prism 5 (GraphPad software, La Jolla, CA, USA). Statistical differences were determined by a non-parametric Wilcoxon (Mann–Whitney) rank-sum test or, in case of more than two samples, a non-parametric Kruskal–Wallis test followed by a Dunn's post hoc test for multiple comparisons, using GraphPad Prism 5 (GraphPad software, La Jolla, CA, USA). Significant levels were reported with $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

II. RESULTS AND DISCUSSION

Toxicity tests

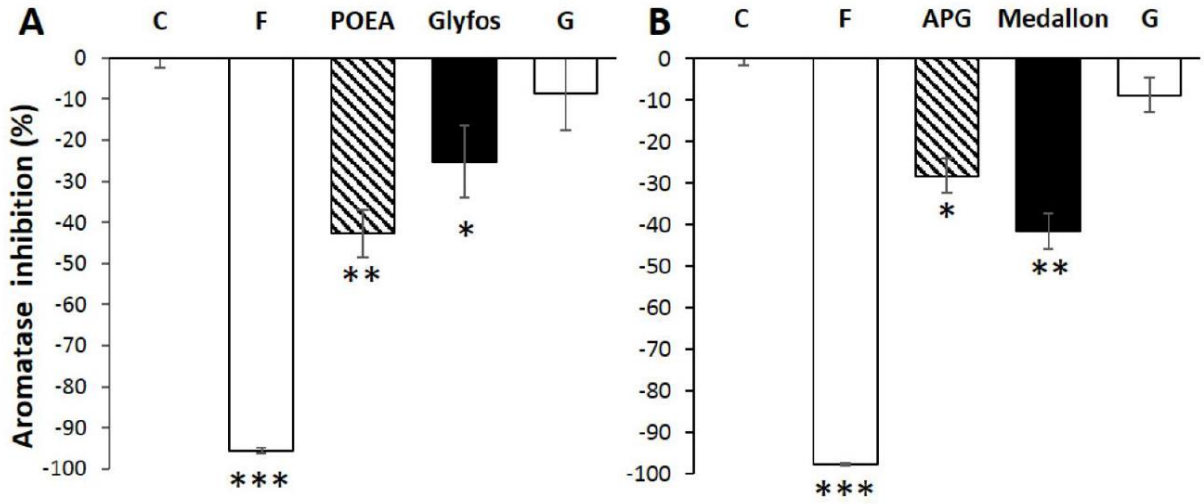
The lowest concentration exerting a significant toxic effect (LOEC) was considered to be the toxicity threshold. The highest concentration without significant cytotoxic effect (NOEC) was also reported. LC_{50}, NOEC, and LOEC values of 7878, 3100, and 4600 mg/L were determined for glyphosate. Glyphosate showed cytotoxic effects at high doses only, whereas the co-formulants or formulations were more toxic by a factor 10-1000 (see the following graph):



The adenylate kinase activity, which was determined as a marker for necrosis, was not influenced by glyphosate when compared to the negative control.

Aromatase activity

Glyphosate had no effect on the aromatase activity when tested at concentrations used in the formulations (16 – 146 mg/L). At very high doses of 3000 ppm a significant aromatase inhibition was observed by glyphosate (-51%; data not shown in article for 3000 ppm which is equivalent to 17.7 mM glyphosate). Nevertheless, according to the authors this is considered not relevant because of the high concentration used. In the following figure the effects of glyphosate (G) on the aromatase activity is exemplary shown (A: POEA, 2.5 ppm; Glyfos, 25 ppm; glyphosate, 16 ppm; B: APG, 120 ppm; Medallon, 300 ppm; glyphosate, 146 ppm). Unlike glyphosate, exposure to formulations and co-formulants did result in aromatase inhibition at non-toxic concentrations.



Discussion

All co-formulants inhibited aromatase and disrupted mitochondrial respiration (and membranes) at higher concentrations. APG and POEA were 15–18 times and 1200–2000 times more cytotoxic than glyphosate, respectively.

III. CONCLUSIONS

Assessment and conclusion by applicant:
Toxicity in JEG3 cells as well as aromatase activity have been investigated. Cells were treated with glyphosate alone, formulations containing glyphosate as active ingredient and co-formulants alone. Glyphosate showed generally low cytotoxicity. An effect on aromatase activity was not observed when cells were treated with glyphosate at non-toxic concentrations. At a higher glyphosate concentration (17.7 mM) a reduction of aromatase activity was seen, which is in accordance with the authors not considered relevant at this high concentration in the test system used. Moreover, such a high concentration is not to be reached at physiologic conditions and therefore, not considered relevant for human health.

The publication on in vitro aromatase activity in human cells shows that, even though it does not follow a defined guideline, the study is well documented and is scientifically sound. Therefore, the study is reliable with restrictions and is relevant for risk assessment.

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

Publication: Defarge *et al*., 2016	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	NA	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	N	
Cytotoxicity tests reported	N	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	NA	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable with restrictions because the glyphosate used was not sufficiently characterized and as a too high, non-physiological concentration had been considered for glyphosate exposure.		

Assessment and conclusion by RMS:

The study is concluded to be reliable (Klimisch Score 1).

Glyphosate itself inhibited aromatase activity at a very high dose of 3000 ppm, which was not considered to be relevant. Glyphosate did not show an effect on aromatase inhibition when tested at non-toxic concentrations which resemble the concentrations used in formulations.

B.6.8.3.17. Public literature – Effect on ovarian folliculogenesis and steroidogenesis

Data point:	CA 5.6
Report author	Ganesan S. *et al*.
Report year	2020
Report title	Absence of glyphosate-induced effects on ovarian folliculogenesis and steroidogenesis
Document No	Reproductive Toxicology, (2020) 96, 156-164 DOI: 10.1016/j reprotox.2020.06.011
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 **Conclusions AGG:** The study is considered to be reliable.

Full summary of the study according to OECD format

Glyphosate (GLY) is an herbicidal active ingredient and both *in vitro* and *in vivo* studies suggest that GLY alters ovarian function. To determine if a chronic GLY exposure model affected steroidogenesis or folliculogenesis in vivo, postnatal day 42 C57BL6 female mice were orally delivered vehicle control (saline) or GLY (2 mg/kg bw) from a pipette tip five days per week for either five or ten weeks. Mice were euthanized at the proestrus stage of the oestrous cycle. GLY exposure did not impact body weight gain, organ weights, or healthy follicle numbers. In addition, GLY exposure did not affect abundance of ovarian mRNA encoding kit ligand (*Kitlg*), KIT proto-oncogene receptor tyrosine kinase (*c-Kit*), insulin receptor (*Insr*), insulin receptor substrate (*Irs1* or *Irs2*) and protein thymoma viral proto-oncogene 1 (AKT) or phosphorylated AKT. Ovarian mRNA or protein abundance of Star, 3β-hydroxysteroid dehydrogenase (*Hsd3b1*), *Cyp11a1* or *Cyp19a* were also not altered by GLY. Circulating 17β-estradiol and progesterone concentration were unaffected by GLY exposure. In conclusion, chronic GLY exposure for five or ten weeks did not affect the ovarian endpoints examined herein.

Materials and methods

Reagents

Glyphosate (CAS # 1071-83-6), 2-β-mercaptoethanol, Tris base, Tris HCL, Sodium chloride, EDTA, SDS, NaF, HEPES and Tween -20 were purchased from Sigma-Aldrich Inc. (St Louis, MO). Ponceau S, Invitrogen iBlot 2NC regular stacks, Pierce BCA protein assay kit, Glycerol, Citric acid, Saline, DAPI nuclear stain and Sodium citrate were obtained from Thermo Fisher Scientific. Mini-PROTEAN TGX™ precast protein gels and protein size markers were purchased from BioRad. RNeasy Mini kit, QIA shredder kit, RNeasy Min elute kit, and Quantitect™ SYBR Green PCR kit were purchased from Qiagen Inc (Valencia, CA). All primers were purchased from the Iowa State University DNA facility (Ames, IA). Antibodies directed against AKT, phosphorylated AKT, CYP11A1, anti-mouse Alexa flour 488 and SignalFire ECL plus chemical luminescence detection kit were from Cell Signaling (Danvers, MA). Antibodies directed against ERα, STAR and donkey anti-rabbit IgG-FITC were from Abcam. Novus Biologicals (Centennial, CO) was the source of antibodies directed against HSD3β and CYP19A1. ELISA kits to measure E_2 and progesterone (P_4) were from DRG International Inc (Springfield, NJ).

Animals

The Iowa State University Institutional Animal Care and Use Committee approved all animal experiments in accordance with NIH guidelines. Postnatal day (PND) 42 C57BL/6 J female mice received vehicle control (saline; n = 20) or glyphosate (GLY; 2 mg/kg/day; n = 20) per os for five days per week for a duration of 5 (n = 10 control; n = 10 GLY) or 10 weeks (n = 10 control; n = 10 GLY). Mice were housed in groups of 3-4 and were provided with ad libitum access to food and drinking water. Body weight was obtained weekly. Vaginal cytological analysis was performed for 10 days in the 5-week exposure study and for 21 days in the 10-week study prior to euthanasia and the length spent in each stage of the oestrous cycle calculated as a percentage of the time analysed. Mice were euthanized at the proestrus phase of their oestrous cycle. Weights of the ovaries, uterus, liver, kidneys and heart were recorded. Blood samples were collected post-euthanasia by cardiac puncture.

Histological analysis
One ovary per animal (n = 10 per treatment) was fixed in 4 % paraformaldehyde for 24 h and transferred to 70 % ethanol and paraffin embedded. Ovaries were sectioned (5 µM thickness) and every 6th section was mounted and stained by hematoxylin and eosin. Healthy oocyte-containing follicles were identified and counted in every 12th section. Unhealthy follicles were distinguished from healthy follicles by the appearance of pyknotic bodies and intense eosinophilic staining of oocytes. Follicles were classified and enumerated as previously described (Flaws *et al*., 1994).

Quantitative reverse transcriptase polymerase chain reaction
Approximately 25 % of each ovary was used for RNA isolation and two ovarian samples were combined (n = 10 ovaries; 5 RNA samples). RNA was isolated using a RNeasy Mini kit and the RNA concentration determined using a Nano Drop spectrometer (λ = 260/280 nm; ND 1000; Nanodrop Technologies Inc., Wilmington, DE). RNA (200 ng) was reverse transcribed to cDNA, followed by quantitative PCR. Primers for specific genes of interest were designed by Primer 3 Input Version (0.4.0) (listed in Table 1). The regular cycling program consisted of a 15 min hold at 95 °C and 45 cycles of denaturing at 95 °C for 15 s, annealing at 58 °C for 15 s, and extension at 72 °C for 20 s at which point data were acquired. Each sample was normalized to 18 s RNA before quantification. Quantification of fold-change in gene expression was performed using the $2^{-\Delta\Delta Ct}$ method.

Table 1: Primer sequences

Gene	Forward Primer	Reverse Primer
c-Kit	ttctccttaggaagcagccc	cctgcttgaatgttggcctt
Kit-lg	tcagtcatagattggagtttgca	tgtatcaaaagggtcgggaca
Akt	tttgttgctgtgtcccatgc	caagtgctaggagaagggct
Star	atgttcctcgctacgttcaag	cccagtgctctccagttgag
Hsd3b1	gctggaaactgtgagcttcc	tgcttcctcccagttgacaa
Cyp11a1	aggtccttcaatgagatcccctt	tccctgtaaatggggccatac
Cyp19a1	atgttcttggaaatgctgaaccc	aggacctggtattgaagacgac
ERa	aattctgacaatcgacgccag	gtgcttcaacattctccctcctc
Erb	tgctccaagggtaggatggac	ctgtgcctcttctcacaagga
Insr	tgtcatcaatgggcagtttg	atcaggttccgaacagttgc
Irs1	ctatgccagcatcagcttcc	ggaggatttgctgaggtcat
Irs2	gaagcggctaagtctcatgg	gacggtggtggtagaggaaa

Western blot analysis
Ovaries (n = 10 per treatment) were homogenized in tissue lysis buffer (containing protease and phosphatase inhibitors) and centrifuged at 10,000 rpm twice for 15 min. The protein concentration of the supernatant was measured using a bicinchoninic acid assay and stored at -80 °C until further use. Protein was separated by SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were blocked for 1 h in 5 % milk in Tris-buffered saline containing tween 20. Membranes were incubated in primary antibodies directed against protein kinase B (AKT; primary - 1:200; secondary - 1:500), phosphorylated AKT (pAKT; primary - 1:200; secondary - 1:200), estrogen receptor alpha (ERα; primary - 1:500; secondary - 1:500), estrogen receptor beta (ERβ; primary - 1:500; secondary - 1:200), steroidogenic acute regulatory protein (STAR; primary - 1:500; secondary - 1:200); 3-β hydroxysteroid dehydrogenase (HSD3β; primary - 1:1000; secondary - 1000), cytochrome P450 isoform 11A1 (CYP11A1; primary - 1:500; secondary - 1:500) and cytochrome P450 isoform 19A1 (CYP19A1; primary - 1:500; secondary - 1:500) overnight at 4 °C with rocking. Following three washes in TTBS (1X) membranes were incubated with species-specific secondary antibody for 1 h at room temperature. Autoradiograms were developed on X-ray films in a dark room following 10 min incubation of membranes with 1X SignalFire™ ECL reagent. Densitometry of the appropriate-sized bands was measured using Image Studio Lite Version 3.1 (LI-COR Biosciences, Lincoln, NE) which eliminates background noise. The sum of the gray values of all the pixels in the selection divided by the number of pixels, or mean grey value was quantified for each membrane using ImageJ software. Membranes were normalized to Ponceau S protein staining in which the entire lane of the transferred protein was quantified to account for loading variation. To ensure antibody specificity, negative control blots for each antibody used were performed in which the membranes were incubated with primary antibody only, secondary antibody only, or normal IgG in place of primary antibody with the inclusion of the appropriate secondary antibody. No protein bands were observed on these control blots indicating the specificity of the protein bands detected and analysed.

Immunofluorescence staining
Paraffin embedded ovaries (n = 5 per treatment) from the 10 week exposure group were serially sectioned (5 μM thick) and every 10^{th} section was mounted. Sections were deparaffinised in xylene and rehydrated with subsequent washes in ethanol. Antigen retrieval was carried out by microwaving sections for 7 min in sodium citrate buffer (1 M, pH 6.1). Sections were blocked in 5 % BSA for 1 h at room temperature. Sections were incubated with primary antibodies directed against pAKT (1:50 dilution), STAR (1:500 dilution), HSD3β (1:500 dilution), CYP11A1 (1:100 dilution), CYP19A1 (1:100 dilution) and ERα (1:200 dilution) overnight at 4 °C. After washing in 1 % PBS, sections were incubated with the appropriate donkey anti-rabbit IgG-FITC (1:200 to 1:500 dilutions) or anti-mouse Alexa flour 488 (1:200 dilution) secondary antibody for 1 h. Slides were counterstained with 4-6-diamidino-2-phenylindole (DAPI) for 5 min. Negative technical controls incubated the ovarian sections omitting both the primary and secondary antibody to ensure specificity of the antibodies. Images were captured using a Leica DMI3000 B fluorescent microscope. Raw internal density of staining was analysed using Image J software. For analysis, 5 ovaries per treatment were assessed with three sections per ovary and 7-10 large follicles per slide analysed.

Steroid hormone quantification
Serum (25 μL; n = 10 per treatment) was added in duplicate per sample to an ELISA plate to measure E_2 or P_4. Plates were incubated for 60-90 min after adding the enzyme conjugate (100-200 μl). The wells were rinsed with wash solution three times, substrate solution added (100-200 μL) and incubated for 15-30 min. The enzymatic reaction was stopped by adding stop solution (50-100 μL) and the signal determined within 10 min using a plate reader at 450 nm absorbance.

Sample identity blinding
The identity of samples used for ELISA and follicle counting were not known to the investigator performing the analysis. In addition, mRNA for PCR and protein samples from mice treated for 10-weeks for western blotting were double blinded - the identity of the samples was unknown to the investigator and after western blots were completed, they were then identified by group for the purpose of statistical analysis. Only after the statistical analysis was completed was group identity revealed.

Statistical analysis
Raw data were analysed by unpaired t-test. All statistical analysis was performed using Prism 5.04 software (GraphPad Software). Statistical significance (*) was defined as $P < 0.05$. A tendency for a statistically meaningful difference was considering if the *P*-value was between 0.05-0.1

Results

Impact of GLY exposure on body weight gain
Body weight gain was determined from the onset to the completion of the dosing period which was either a duration of 5 or 10 weeks. As anticipated, mice gained weight with aging, however, there was no impact of GLY exposure on body weight gain at either time point (Figure 1).

Effect of GLY exposure on the estrous cyclicity
In mice exposed to GLY for either duration, no impact of GLY exposure on the percentage time spent at any stage of the oestrous cycle was observed, though there was a tendency (P <0.1) for increased time spent in estrus and reduced time spent in metestrus/diestrus after 5 weeks of GLY exposure (Figure 2).

Endocrinological impact of GLY exposure
Mice were euthanized at the proestrus stage of the oestrous cycle, thus E_2 (Figure 3A) levels were higher than P_4 (Figure 3B). There was no effect of 5 or 10 weeks of GLY exposure on circulating E_2 (Figure 3A). No impact of GLY exposure on serum P_4 was noted at either timepoint (Figure 3B).

Relative organ weight effects of GLY exposure
There was no effect of either duration of GLY exposure on the relative weight of the heart (Figure 4A,F), liver (Figure 4B,G), kidneys (Figure 4C,H), or uterus (Figure 4D,I). In addition, neither 5 weeks (Figure 4E) or 10 weeks of GLY exposure (Figure 4J) affected ovarian weight, although a tendency (P <0.1) towards a reduction in ovarian weight after 10 weeks of exposure was observed.

Impact of GLY exposure on ovarian follicle number
GLY exposure for 5 (Figure 5A) or 10 weeks (Figure 5B) did not influence the number of ovarian primordial, primary, secondary or antral follicles. There was also not a different between CT and GLY treated mice at either 5 or 10 weeks in total follicle number or the percentage of follicles per developmental stage within the ovary. Corpora lutea were evident, thus, there were not a defect in ovulation noted and there was no different in the number of CL per ovary due to GLY exposure (data not shown and Figure 5C-F).

Effect of GLY exposure on ovarian mRNA encoding genes involved in ovarian folliculogenesis and steroidogenesis
GLY exposure did not impact abundance of ovarian mRNA encoding the PI3K members kit ligand (*Kitl*), KIT proto-oncogene receptor tyrosine kinase (*c-Kit*), or protein kinase b (*Akt*) after 5 (Figure 6A) or 10 (Figure 6D) weeks. GLY exposure also did not alter ovarian mRNA abundance of steroidogenic acute regulatory protein (*Star*), 3β-hydroxysteroid dehydrogenase (*Hsd3b*), cytochrome P450 (*Cyp*) *11a1* and *Cyp19a* after 5 (Figure 6B) or 10 weeks (Figure 6E) of exposure. In addition, there was no effect of GLY exposure on mRNA encoding the ovarian insulin receptor (*Insr*), insulin receptor substrate 1 (*Irs1*) or *Irs2*, estrogen receptor (*Er*) alpha (*Erα*) or *Er* beta (*Erβ*) mRNA abundance at either 5-weeks (Figure 6C) or 10-weeks (Figure 6F) post-exposure.

GLY exposure impact on ovarian folliculogenesis and steroidogenesis protein abundance
Using western blot analysis to interrogate changes to ovarian proteins involved in folliculogenesis or steroidogenesis, no impacts of 5 (Figure 7A,B) or 10 (Figure 7C,D) weeks of GLY exposure on ERα, ERβ, AKT, pAKT, STAR, HSD3β, CYP11A1, or CYP19A1 protein abundance were detected. Use of immunofluorescence staining in ovaries from the 10-week exposure group identified localization of pAKT (Figure 8A-C), STAR (Figure 8D-F), HSD3β (Figure 8G-I), CYP11A1 (Figure 8J-L), CYP19A1 (Figure 8M-O) and ERα (Figure 8 P–R) but, with the exception of a tendency for a reduction in HSD3B and CYP19A1 (P <0.1); Figure 9A,B). there was no difference between saline- and GLY-treated mice in the internal density of immunostaining for these proteins.

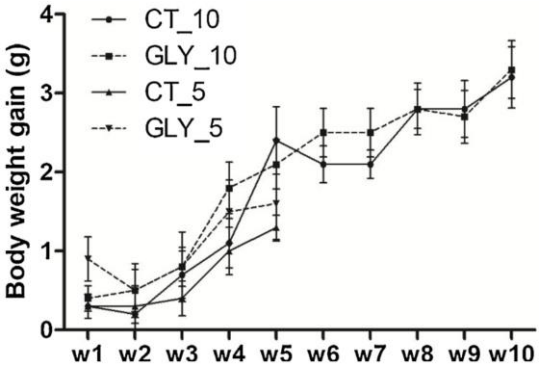


Figure 1: Effect of glyphosate on body weight. Female C57Bl/6 mice were exposed to saline vehicle control (CT) or GLY (2 mg/Kg) per os from a pipette tip five days per week for 5 or 10 weeks. Body weight was measured from day 0 of dosing to end of 5 or 10 weeks of dosing. Values are expressed as average body weight gained every week ± SEM; n = 10. Mice exposed to saline (CT) for 5 weeks indicated by solid line with triangle data point marker; Mice exposed to GLY for 5 weeks indicated by dashed line with triangle data point marker; Mice exposed to saline (CT) for 10 weeks indicated by solid line with circle data point marker; Mice exposed to GLY for 10 weeks indicated by dashed line with square data point marker.

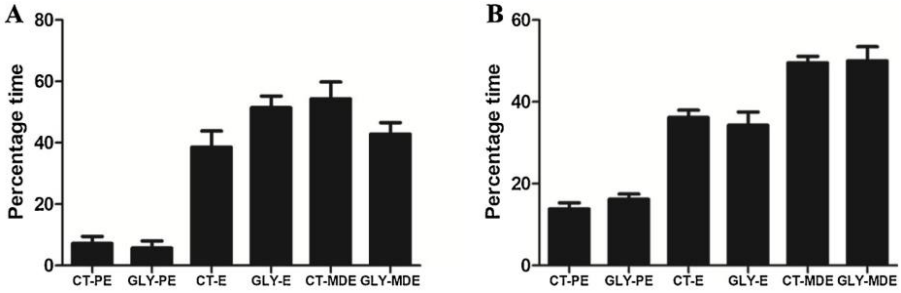


Figure 2: Effect of GLY exposure on percentage time spent at stages of oestrous cycle. Female C57Bl/6 mice were exposed to saline vehicle control (CT) or GLY (2 mg/Kg) per os from a pipette tip five days per week for (A) 5 or (B) 10 weeks. The percentage time spent at stages of oestrous cycle were calculated: proestrus = PE; oestrus = E; metestrus and diestrus = MDE. Data points represent mean +/− SEM. Statistical analysis was performed on raw data.

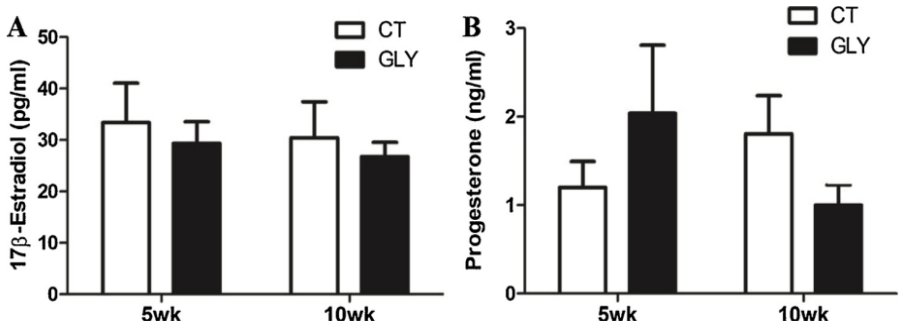


Figure 3: GLY exposure effect on circulating E_2 and P_4. Female C57Bl/6 mice were exposed to saline vehicle control (CT) or GLY (2 mg/Kg) per os from a pipette tip five days per week for 5 or 10 weeks. Circulating (A) E_2 and (B) P_4 were measured by ELISA. Data points represent mean +/- SEM.

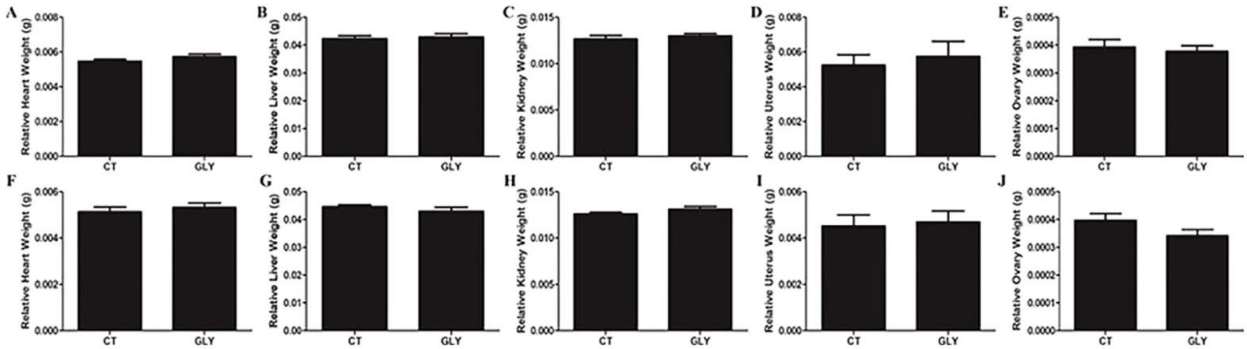


Figure 4: Relative organ weight impacts of GLY exposure. Female C57Bl/6 mice were exposed to saline vehicle control (CT) or GLY (2 mg/Kg) per os from a pipette tip five days per week for (A-E) 5 or (F-J) 10 weeks. Organ weights (g) were collected post-euthanasia and normalized to body weight: (A,C) Heart, (B,G) Liver, (C,H) Kidney, (D,I) Uterus and (E,J) Ovary weight. Data points represent mean +/- SEM.

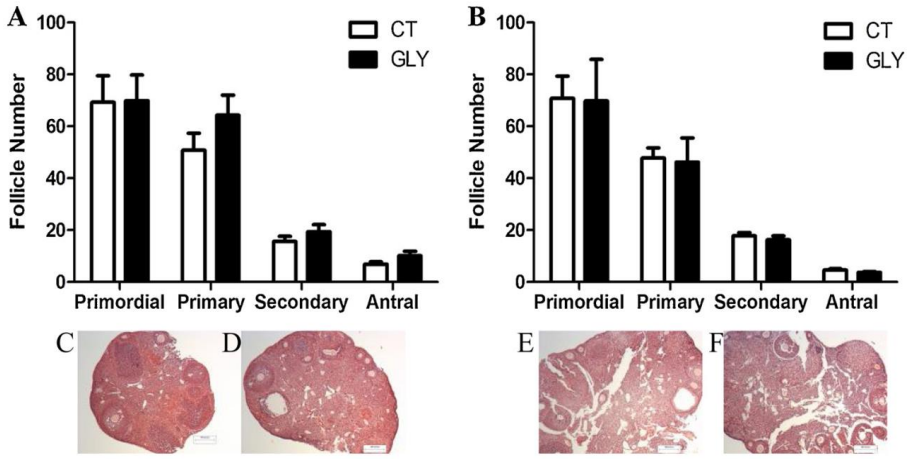


Figure 5: Effect of GLY exposure on ovarian follicle number. Female C57Bl/6 mice were exposed to saline vehicle control (CT) or GLY (2 mg/Kg) per os from a pipette tip five days per week for (A) 5 or (B) 10 weeks. Ovaries were sectioned, stained with haemotoxylin and eosin and follicular stages classified and counted as primordial, primary, secondary, and antral. Data points represent mean +/- SEM. Representative (C) CT 5 week, (D) GLY 5 week, (E) CT 10 week and (F) GLY 10 week ovaries are provided.

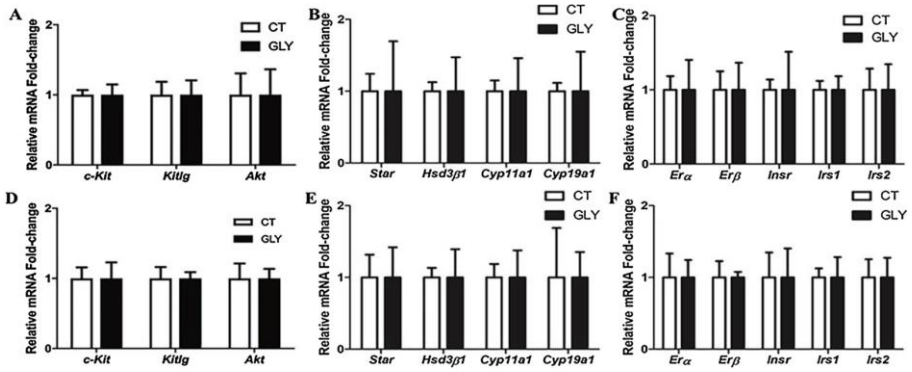


Figure 6: Impact of GLY exposure on ovarian mRNA abundance. Female C57Bl/6 mice were exposed to saline vehicle control (CT) or GLY (2 mg/Kg) per os from a pipette tip five days per week for (A-C) 5 or (D-F) 10 weeks. Ovaries were homogenized and mRNA isolated for qRT-PCR to analyse relative abundance of genes involved in (A,D) PI3K signaling; (B,E) Steroidogenesis; and (C,F) Endocrine signaling. Data points represent fold change relative to CT treated ovaries +/- SEM.

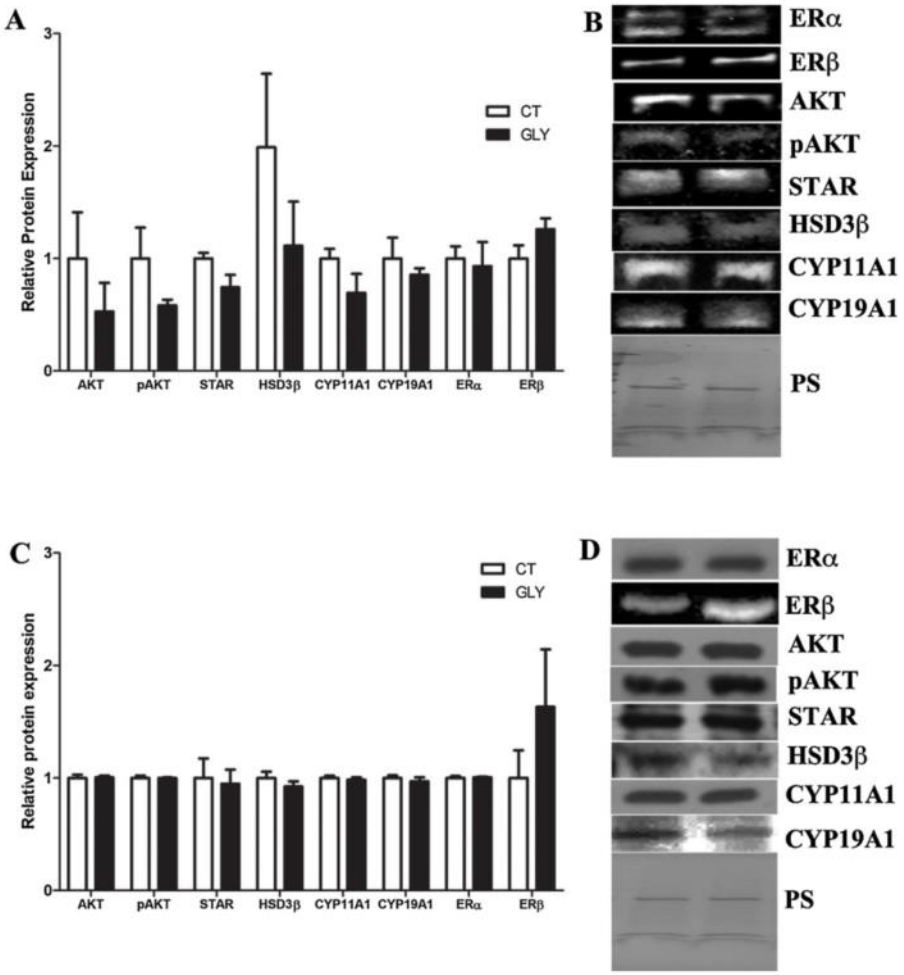


Figure 7: Consequence of GLY exposure on ovarian protein abundance. Female C57Bl/6 mice were exposed to saline vehicle control (CT) or GLY (2 mg/Kg) per os from a pipette tip five days per week for (A,B) 5 or (C,D) 10 weeks. Ovaries were homogenized and western blotting performed to quantify protein abundance of AKT, pAKT, STAR, HSD3B, CYP11A1, CYP19A1, ERA, and ERB. (A,C) Bars represent mean +/- SEM. (B,D) Representative images of western blots for each protein with ponceau S staining represented as PS.

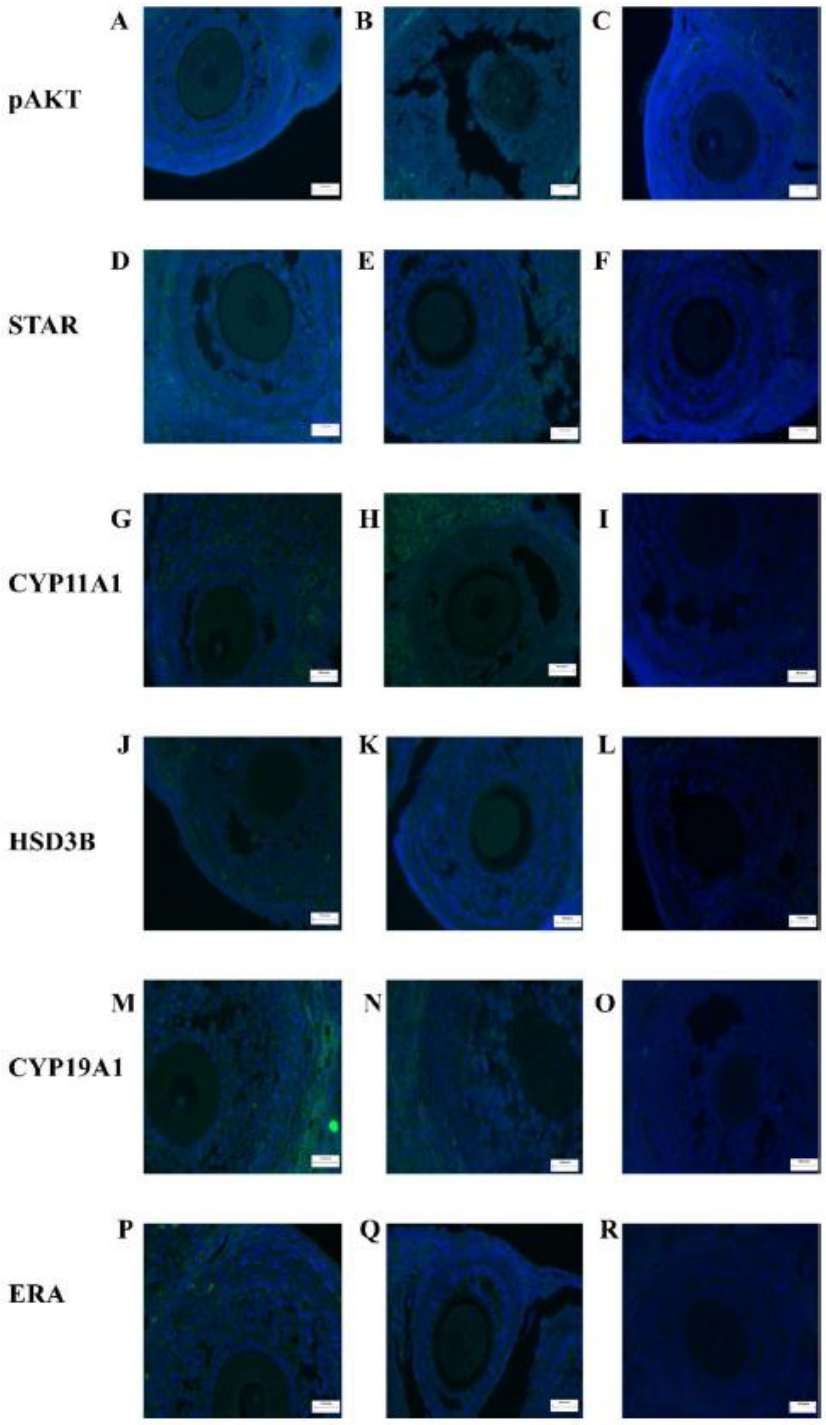


Figure 8: Effect of GLY exposure on ovarian protein localization in large pre-antral follicles. Female C57Bl/6 mice were exposed to saline vehicle control (CT) or GLY (2 mg/Kg) per os from a pipette tip five days per week for 10 weeks. Immunostaining was performed only in ovaries from the 10 week exposure. Ovaries were prepared for immunofluorescence staining to localize and quantify protein abundance of (A-C) pAKT, (D-F) STAR, (G-I) CYP11A1, (J-L) HSD3B, (M-O) CYP19A1 and (P-R) ERA. Blue staining represents DNA and green staining indicates protein of interest. Scalebar = 100 microns.

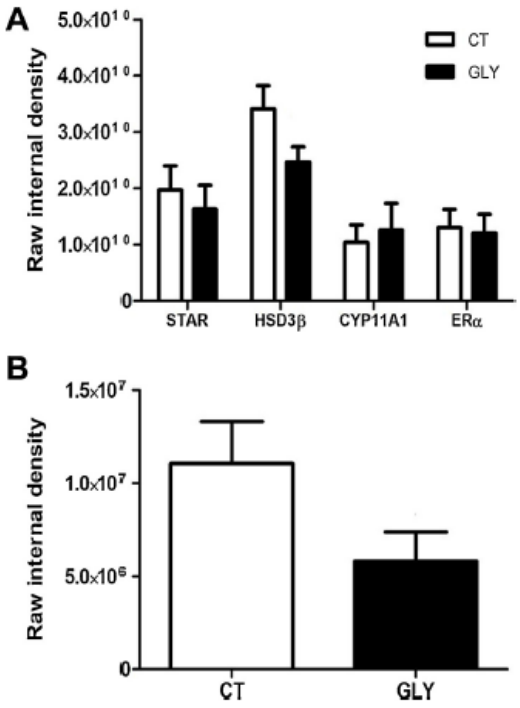


Figure 9: Effect of GLY exposure on ovarian protein quantification in large preantral follicles. Female C57Bl/6 mice were exposed to saline vehicle control (CT) or GLY (2 mg/Kg) per os from a pipette tip five days per week for 10 weeks. Ovaries that were immunofluorescence stained were analyzed for abundance of (A) STAR, HSD3B, CYP11A, and ERA. (B) Quantification of CYP19A1. Bars represent mean internal density +/- SEM.

Conclusions

Taken together, this study determined absence of GLY-induced alterations to the reproductive endpoints measured. After both 5 and 10 weeks of GLY exposure, relative to the saline vehicle control treated mice, any impacts of GLY on endpoints measured was largely absent. This study involved sample deidentification to remove any unintentional or perceived bias, was chronic in nature and performed at a conservative dosage (relevant to human exposure). In summary, the findings of the current study do not support that chronic, oral GLY at a dose considered non-hazardous alters oestrous cyclicity, follicle number, circulating ovarian steroid hormone levels and ovarian intracellular signalling in adult female mice.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In vivo study on post natal day 42, glyphosate administered to C57BL/6 J female mice at 0 or 2 mkd (10 mice/dose). Cyclicity, follicle number, circulating ovarian steroid hormone levels and ovarian intracellular signaling parameters were tested in adult female mice during 5 or 10 weeks. Glyphosate exposure for five or ten weeks did not affect the ovarian and endocrine endpoints examined.

The article is classified as reliable with restrictions for the following reasons: only 1 dose tested (no dose relationship can be evaluated), purity of glyphosate is not clear, method of analysis for analysis test media & no validation of the analytical method was described, no GLP status stated, no OECD guideline followed. Although no HCD were available in order to compare with the equivalent concurrent controls and test groups results, the results were negative and therefore did not require HCD to interpret or provide context to any findings.

Reliability Criteria by applicant: *In Vivo* Toxicology Studies		
Publication: Ganesan S. *et al*. 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		

Reliability Criteria by applicant: *In Vivo* Toxicology Studies		
Publication: Ganesan S. *et al*. 2020	**Criteria met? Y/N/?**	**Comments**
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	Not stated
Study completely described and conducted following scientifically acceptable standards	Y	Use of control group, 10 female mice/group, 1 dose + 1 negative control, statistical analysis performed, no randomisation of animals mentioned, no HCD
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate No Purity, no storage condition stated
Only glyphosate acid or one of its salts is the tested substance	N	Glyphosate
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	C57BL/6 J mice, age, origin stated but initial BW not included
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Per os
Dose levels reported	Y	0 and 2 mg/kg/d; 5 days/week for 5 and 10 weeks
Number of animals used per dose level reported	Y	10 mice/group 5 weeks (n = 10 control; n = 10 GLY) and 10 weeks (n = 10 control; n = 10 GLY)
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	N	Only 1 dose tested
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	only 1 dose tested (no dose relationship can be evaluated), purity of glyphosate is not clear, method of analysis for analysis test media & no validation of the analytical method was described, no GLP status stated, no OECD guideline followed. Although no HCD were available in order to compare with the equivalent concurrent controls and test groups results, the results were negative and therefore did not require HCD to interpret or provide context to any findings.

Reliability Criteria by applicant: *In Vivo* Toxicology Studies		
Publication: Ganesan S. *et al*. 2020	**Criteria met? Y/N/?**	**Comments**
Not reliable	N	

Assessment and conclusion by RMS:
The effect of glyphosate was investigated in an *in vivo* study. C57BL/6 J female mice (PND 42) received either 2 mg/kg bw glyphosate per os for 5 or 10 weeks (5 doses per week); the corresponding control animals received saline. Each dose group consisted of 10 animals. Body weight, cyclicity, follicle number, circulating ovarian steroid hormone levels and ovarian intracellular signalling parameters (representative for folliculogenesis and steroidogenesis) were tested in all animals during and after the dosing period. No difference between the treated and control animals were seen for any parameter following the 5 or 10 week exposure period.

The applicant lists a few reasons why the study should be considered reliable with restrictions (see above). While the RMS agrees that these reasons are indeed correct, the overall reliability of the study is not doubted. Therefore the study is considered reliable (Klimisch score 1).

B.6.8.3.18. Public literature – Effect on human endometrial cells via estrogen receptor pathway

Data point:	CA 5.8.3
Report author	Gastiazoro M.P. *et al.*
Report year	2020
Report title	Glyphosate induces epithelial mesenchymal transition-related changes in human endometrial Ishikawa cells via estrogen receptor pathway
Document No	Molecular and cellular endocrinology, (2020), Vol. 510, Art. No. 110841
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 **Conclusions AGG:** The study is considered to be reliable.

Full summary of the study according to OECD format

Ishikawa cells were exposed to glyphosate (Gly) (0.2 μM and 2 μM) or 17β-estradiol (E2: 10^{-9} M). Gly increased cell migration and invasion ability compared to vehicle, as did E2. Moreover, a down regulation of E-cadherin mRNA expression was determined in response to Gly, similar to E2-effects. These results show that Gly promotes epithelial mesenchymal transition (EMT)-related changes in Ishikawa cells. When an ER antagonist (Fulvestrant: 10^{-7} M) was co-administrated with Gly, all changes were reversed, suggesting that Gly might promote EMT-related changes via ER-dependent pathway. The results are interesting evidences of Gly effects on endometrial cancer progression via the ER-dependent pathway.

Materials and methods

Substances
All reagents and chemicals were of analytical grade. Glyphosate (CAS N° 1071-83-6) used as the PESTANAL® analytical standard (purity grade ≤ 100%) and 17β-estradiol (E2; purity ≥ 98%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Fulvestrant (ICI 182,780; purity ≥ 99%) was purchased from Tocris Bioscience (Minneapolis, Minnesota, USA).

Cell culture

The human endometrial adenocarcinoma cell line Ishikawa was provided by Masato Nishida, Department of Obstetrics and Gynecology, University of Tsukuba. These cells were obtained from an endometrial adenocarcinoma of a 39-year-old woman in 1985 by Nishida, and established as ER- and PR-positive cell line. The cells were cultured in Dulbecco's modified Eagle's medium F12 (DMEM/F12) (Biowest, Germany) supplemented with 10% fetal bovine serum (FBS) and 1% Insulin-Transferrin-Selenium A (ITS) (Gibco-BRL, Grand Island, NY, USA) and maintained in culture 75 cm^2 flask at 5% CO2 and 37 °C. The medium was replaced every 48 h. Ishikawa cells were grown to 80% of confluence and enzymatically detached by trypsin and (0.05%) EDTA at 37 °C.

Treatment conditions
The concentrations in the experiment were chosen based on the reference dose (RfD) of 1 mg of glyphosate/kg b.w. per day established by U.S. EPA and it was in a range between 0.2 µM and 200 µM (0.2 µM, 2 µM, 20 µM and 200 µM), an interval of concentrations that include the RfD. A 1 mM Gly stock solution was prepared with ethanol and different volumes were added during the treatment to achieve the corresponding Gly concentration. In detail, to final Gly concentration 0.2 µM in a 6-well plate with 2 ml media per well, 0.4 µl of Gly stock solution was added; to final Gly concentration 2 µM was added 4 µl; to final Gly concentration 20 µM was added 40 µl of Gly stock solution and to final Gly concentration 200 µM was added 400 µl of Gly stock solution. As a positive control estradiol (E2) at 10^{-9} M was used. ER antagonist Fulvestrant was tested at concentration of 10^{-7} M. Dimethylsulfoxide (DMSO) was used as vehicle for E2 and Fulvestrant and added in a way that the DMSO concentration in the test did not exceed 0.1%. Ethanol was used as vehicle for Gly and added in a way that ethanol concentration in the test did not exceed 2%. No difference between DMSO and ethanol was detected among assays. For that reason, vehicle is shown as a unique condition in the results. To carry out the treatments, the cells were seeded in a 6-well plate for 24 h to establish adherent monolayer after that the corresponding drug-treatment was added depending on the assay. The medium with the corresponding amount of Gly, E2, or Fulvestrant was renewed every 24 h.

Trypan blue dye exclusion assay
The authors determined the viability of Ishikawa cells after treatment with selected concentrations of Gly by trypan blue dye exclusion assay. The aim was to determine whether the assessed concentrations altered cell viability in a significant way. Four concentrations of Gly were tested, Gly 0.2: 0.2 µM, Gly 2: 2 µM, Gly 20: 20 µM and Gly 200: 200 µM. The exclusion criterium was to reject the concentrations that cause a viability ≤80% and show statistical significant difference respect to vehicle.

Scratch-wound healing assay
The Ishikawa cells were seeded in 6-well plates at density of 200,000 cells/well with DMEM/F12 - FBS 10% - ITS 1% medium and incubated at 5% CO_2 and 37 °C with vehicle, E2 or different Gly concentrations with/without addition of Fulvestrant for 24 h. Then the monolayers were scratched using a sterile 200 µl micropipette tip, and washed with PBS twice to remove the detached cells. The different treatments were renewed and the DMEM/F12 - FBS 10% - ITS 1% medium was replaced with DMEM/F12 - FBS 1% - ITS 1% medium. The cells were incubated during a 72-h period, renewed the media and treatments every 24 h. The 72-h period was selected based on the frame time of incubation on which only under optimal condition (positive control) be close to closure the scratch. In order to measure the percentage of wound closure area by migrating cells, 8–10 images (from each treatment group) of the wounded cell monolayers were taken at 0 and 72 h after scratching at 40X using an Olympus CK40 Inverted Microscope (Olympus, Japan) coupled with a camera Canon Power Shot G9 (Canon, Japan). The average area of wound covered was calculated as percentage of wound closure after 72 h = (uncovered area at 0 h – uncovered area at 72 h)/uncovered area at 0 h x 100. The uncovered areas were determined using Image J software. The results were expressed as % wound closure after 72 h relative to vehicle.

Transwell invasion assay
The invasion assay was conducted using Falcon cell culture insert 8 µm pore size placed into a 24-well plate. The inserts were uniformly coated with Matrigel® basement membrane matrix (Corning Life Science, U.S.) for 1 h at 37 °C before cells were added. Cells were treated with vehicle, E2 or different Gly concentrations with/without addition of Fulvestrant for 24 h and then, harvested by trypsinization. Cells (1×10^5 cells/well) were seeded into the upper chamber in 200 µl of DMEM/F12-FBS 1% - ITS 1% medium, while the bottom of the chamber was incubated with 750 µl DMEM/F12-ITS 1% medium containing FBS 20% as a chemoattractant. After 48 h of incubation, cells migrating from the top chamber to the lower surface of the insert, as a result of cell invasion through Matrigel®, were fixed 3 min with formaldehyde 4% and 20 min with methanol. Finally, the cells were stained 20 min with crystal violet 0.1%. To quantify the number of invasive

cells, all the slides with stained cells were photographed at 40X using an Olympus CK40 Inverted Microscope (Olympus, Japan) coupled with a camera Canon Power Shot G9 (Canon, Japan). Images were analysed using the cell counting tool in Image J software. The number of invasive cells after 48 h was determined. The results were expressed as number of invasive cells after 48 h relative to vehicle.

RNA extraction, reverse transcription and quantitative polymerase chain reaction (RT-qPCR)
The Ishikawa cells were seeded in 6-well plates with DMEM/F12 - FBS 10% - ITS 1% medium and incubated at 5% CO_2 and 37 °C with vehicle, E2 or different Gly concentrations with/without addition of Fulvestrant for 24 h. Total RNA from cultured cells was isolated after treatment using Tri-Fast™ (Peqlab VWR, Germany) according to the manufacturer's instructions. Genomic DNA contamination was removed by enzymatic digestion (RQ1 DNase, Promega, U.S.) and checked by PCR. First-strand cDNA synthesis was performed by mixing 2 µg of digested RNA with MMLV reverse transcriptase (Promega, U.S.) and Oligo (dT) 12–18 primers (Eurofins MWG Operon, Germany). Quantitative real-time PCR was applied for cDNA amplification with SybrGreen I as the detection dye using the iCycler iQ™ Real-Time PCR Detection System (BioRad, U.S.). Product purity was confirmed by dissociation curves and random samples were subjected to agarose gel electrophoresis. Controls containing no template DNA were included in all assays, yielding no consistent amplification. To quantify the expression relative to the vehicle-treated cells, the ΔΔCT method was used. The expression of E-cadherin and Vimentin were normalized to the housekeeping gene ribosomal protein S18 (Rps 18). All PCR reactions were conducted from three independent cell culture experiments.

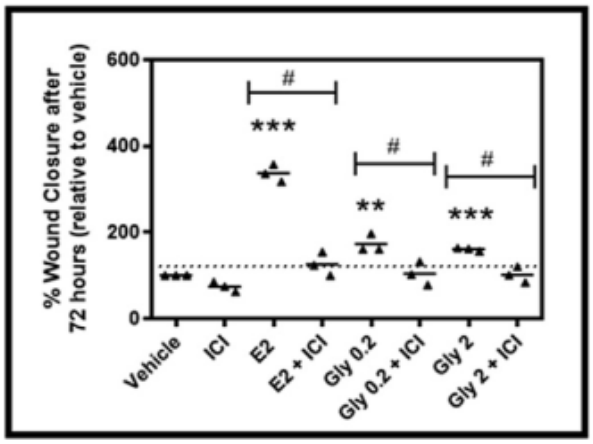
Statistical analysis
Data are presented as the mean ± SEM of three independent experiments. Statistical differences were determined by one-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test using the Graph-Pad Prism software Version 5.03 (San Diego, CA, USA). P-values < 0.05 were regarded as statistically significant.

Results

Effects of Glyphosate on cell viability of Ishikawa endometrial cancer cell
The two highest tested concentrations (20 µM and 200 µM) showed statistical significant difference respect to vehicle and the cell viability was lower than 80%. For that reason, these two concentrations were excluded. Contrary, 0.2 µM and 2 µM did not show differences respect to vehicle and the cell viability was kept above 80%. Based on these results, it was ensured that the cell survival is not altered by the two lower concentrations and it was decided to perform all the experiments with 0.2 µM and 2 µM.

Glyphosate induced Ishikawa endometrial cancer cell migration via estrogen receptor pathway
Migratory activity of Ishikawa cells was measured by scratch-wound healing assay. Fig. 6.8.3.18-1 shows the effects of Gly exposure on the percentage of wound closure after 72 h (relative to vehicle) evidencing that Gly 0.2 µM and Gly 2 µM promoted the migration of Ishikawa cells as did E2. In addition, there was no significant difference between Gly 0.2 µM and Gly 2 µM, for that reason migratory effect no dose dependency could be revealed. After Ishikawa cells were treated with E2 plus Fulvestrant or Gly plus Fulvestrant, the percentage of wound closure after 72 h did not change compared to the vehicle control (Fig. 6.8.3.18-1). In other words, the E2 or Gly effects were reversed by Fulvestrant.

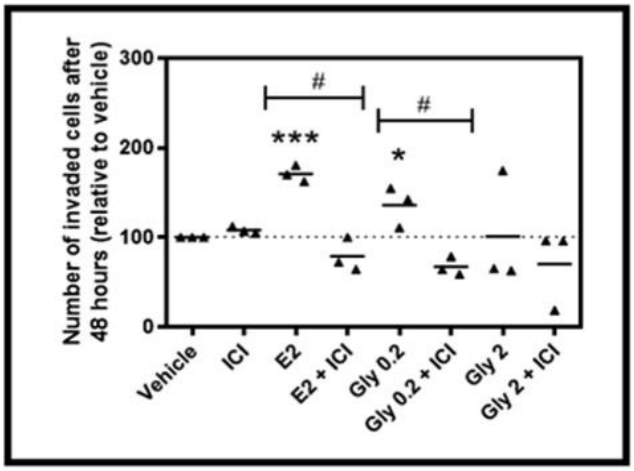
Figure 6.8.3.18-1: Effects of Gly and Fulvestrant (ICI 182,780) treatment on migration ability of Ishikawa endometrial cancer cell tested via the Scratch-Wound Healing Assay. Results are presented as percentage of wound closure after 72 h relative to the vehicle control. Data expressed as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001. # indicates means values of the mixture of ICI 182,780 with E2, Gly 0.2 or Gly 2 were significantly reduced from E2, Gly 0.2 or Gly 2 alone.



Glyphosate induces Ishikawa endometrial cancer cell invasion via estrogen receptor pathway

To investigate the invasiveness of Ishikawa cells, transwell invasion assay was conducted. Fig. 6.8.3.18-2 shows the effects of Gly exposure on the number of invaded cells compared to vehicle, evidencing that Gly 0.2 μM promoted the invasion of Ishikawa cells as did E2. Gly 2 μM did not show statistical difference. After Ishikawa cells were treated with E2 plus Fulvestrant or Gly plus Fulvestrant, the number of invasive cells after 48 h did not change compared to the vehicle control, indicating that Fulvestrant attenuated the Gly 0.2 μM and E2 effects.

Figure 6.8.3.18-2: Effects of Gly and Fulvestrant (ICI 182,780) treatment on invasion ability of Ishikawa endometrial cancer cell. Invasion ability was evaluated by the Transwell Invasion Assay with Matrigel®. The results are expressed as number of invasive cells after 48 h and presented as a percentage of the vehicle. Data expressed as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

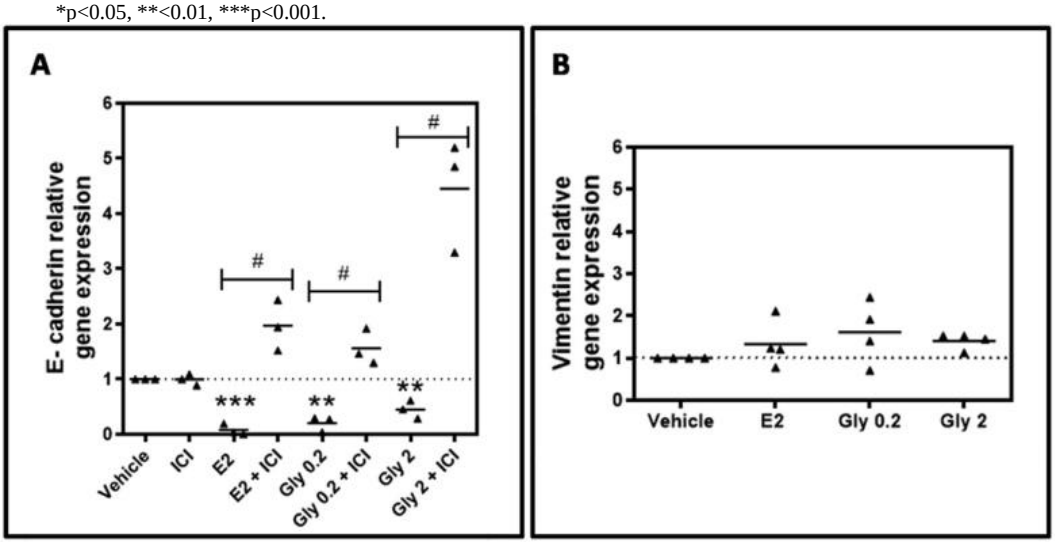


Glyphosate down-regulates E-Cadherin mRNA expression in Ishikawa endometrial cancer cells via estrogen receptor pathway

E-cadherin and vimentin mRNA expression was determined 24 h after the application of different concentrations of Gly or E2 (10^{-9} M). Fig. 3A shows that Gly down regulated E-cadherin mRNA expression of Ishikawa cells, as did E2. Gly 0.2 μM produced a more pronounced effect on down regulation of E-cadherin mRNA expression in comparison with Gly 2 μM (Fig. 3A). Vimentin mRNA expression showed no changes with any of the treatments (Fig. 3B). After combinatorial treatment with E2 plus Fulvestrant or Gly plus Fulvestrant, E-cadherin mRNA expression was restored and even up regulated in respect to the vehicle (Fig. 3A). This up regulation has higher potency to Gly 2 μM than E2 and Gly 0.2 μM.

Figure 3: Effects of Gly and Fulvestrant treatment on E-Cadherin gene expression (A) of Ishikawa endometrial cancer cell. Gly treatment effects on Vimentin gene expression (B) in Ishikawa endometrial cancer cell. Relative gene expression was

quantified by RT-qPCR. The samples were normalized to the housekeeping gene RPS18. Data expressed as mean ± SEM. *p<0.05, **<0.01, ***p<0.001.



Conclusions

The results show that Gly promotes EMT process through the down regulation of E-Cadherin and increase cell migration and invasion abilities. Moreover, all changes could be prevented by the application of Fulvestrant (ER antagonist). According to our knowledge, these are the first pieces of evidence showing Gly effects on endometrial cancer cell progression via the ER-dependent pathway. The findings, in accordance with others, suggest that Gly might increase the risk of aggravating the disease for cancer patients.

Further studies are needed to shed light on the in vivo effects of Gly on the EMT process and cancer metastasis to contribute to the knowledge about carcinogenic potential of the herbicide.

Assessment and conclusion by applicant:

Ishikawa endometrial cancer cells were treated with glyphosate at 0.2 µM and 2 µM. Glyphosate caused cell migration, invasion ability and down regulated E-cadherin mRNA expression. 17ß-estradiol, which was included as a positive control caused similar epithelial mesenchymal transition related changes, while treatment with fulvestrant (estrogen receptor antagonist) reversed the effects caused by glyphosate. The findings suggest that glyphosate has the ability to trigger the estrogen receptor-dependent pathway.

The relevance to human health risk assessment of this unvalidated *in vitro* research model in an immortal adenocarcinoma cell line containing estrogen and progesterone receptors is not clear. The results contradict a number of higher tier studies conducted across a variety of test systems.

The article is classified as reliable with restrictions for the following reasons: glyphosate was tested at two different concentrations only, no test guideline was used and no historical control data were provided in order to compare with the equivalent concurrent controls and test groups results. Further, the study was not performed according to GLP.

Reliability criteria by applicant for *in vitro* toxicology studies		
Publication: Gastiazoro M.P. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	Not stated
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported	Y	Glyphosate

Reliability criteria by applicant for *in vitro* toxicology studies		
(i.e. purity, source, content, storage conditions)		(purity ≤ 100%)
Only glyphosate acid or one of its salts is the tested substance	N	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Ishikawa cells
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	Not necessary
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.2 µM and 2 µM
Cytotoxicity tests reported	Y	Viability was assessed with tryptan blue dye exclusion assay
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	Y	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	Glyphosate was tested at two different concentrations only, no test guideline was used and no historical control data were provided in order to compare with the equivalent concurrent controls and test groups results. Further, the study was not performed according to GLP.
Not reliable	N	

Assessment and conclusion by RMS:

In this *in vitro* study, Ishikawa human endometrial cancer cells were treated with glyphosate at 0.2, 2, 20, and 200 µM. A scratch-wound healing assay, a transwell invasion assay, and the quantification of E-cadherin and vimentin expression was performed with cells treated at 0.2 and 2 µM only as cell viability was below 80% at the two higher concentrations. E2 served as positive control. Besides, the same experimental conditions were conducted in which fulvestrant (also known as ICI 182,780; an estrogen receptor antagonist) was added as well. Glyphosate and the positive control E2 induced Ishikawa endometrial cancer cell migration and invasion, as well as the downregulation of E-cadherin mRNA expression. Since these observations were reversed by the addition of fulvestrant, the results indicate that these processes are estrogen receptor-dependent. The results also indicate that glyphosate and E2 caused epithelial-mesenchymal-transition-related changes, being an indicator for initiation of metastasis.

The applicant lists a few reasons why the study should be considered reliable with restrictions (see above). While the RMS agrees that these reasons are indeed correct, the overall reliability of the study is not doubted. Therefore the study is considered reliable (Klimisch score 1).

B.6.8.3.19. Public literature – Effect on testosterone synthesis inhibition in TM3 cells

Data point:	CA 5.8.3
Report author	Xia Y. *et al*.
Report year	2020
Report title	The endoplasmic reticulum stress and related signal pathway mediated the glyphosate-induced testosterone synthesis inhibition in TM3 cells
Document No	Environmental Pollution, (2020) 260, 113949

	DOI: 10.1016/j.envpol.2020.113949
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 **Conclusions AGG:** The study is considered to be reliable with restrictions.

Full summary of the study according to OECD format

The authors investigated the effects of glyphosate on testosterone secretion and the role of endoplasmic reticulum (ER) stress in the process in TM3 cells (murine Leydig cell line). The effects of glyphosate at different concentrations on the viability of TM3 cells were detected by the CCK8 method. The effect of glyphosate exposure on testosterone secretion was determined by enzyme-linked immunosorbent assay (ELISA). The expression levels of testosterone synthases and ER stress-related proteins were detected by Western blot and Immunofluorescence stain. Results showed that exposure to glyphosate at concentrations below 200 mg/L had no effect on cell viability, while the glyphosate above 0.5 mg/L could inhibit the testosterone secretion in TM3 cells. Treatment of TM3 cells with glyphosate at 5 mg/L not only reduced the protein levels of testosterone synthase StAR and CYP17A1, inhibited testosterone secretion, but also increased the protein level of ER stress molecule Bip and promoted the phosphorylation of PERK and eIF2α. Pre-treatment cells with PBA, an inhibitor of ER stress, alleviated glyphosate-induced increase in Bip, p-PERK, and p-eIF2α protein levels, meanwhile rescuing glyphosate-induced testosterone synthesis disorders. When pre-treatment with GSK2606414, a PERK inhibitor, the glyphosate-induced phosphorylation of PERK and eIF2α was blocked, and the glyphosate-inhibited testosterone synthesis and secretion were also restored. Overall, the authors suggest that glyphosate can interfere with the expression of StAR and CYP17A1 and inhibit testosterone synthesis and secretion via ER stress-mediated the activation of PERK/eIF2α signalling pathway in Leydig cells.

Materials and methods

Chemicals and antibodies
The following chemicals and antibodies were used in this study: Cell Counting Kit (CCK)-8 (Dojindo Molecular Technologies, Shanghai, China), Testosterone ELISA Kit (Mlbio, Shanghai, China), GSK2606414 (Absin, Shanghai, China), 4-phenylbutyric acid (PBA), goat anti-rabbit IgG-TRITC antibody and mouse anti-3β-HSD antibody (Santa Cruz Biotech, Santa Cruz, CA, USA), rabbit anti-Bip antibody, rabbit anti-PERK antibody, rabbit anti-PERK (phosphor T980) antibody, rabbit anti-eIF2α antibody, rabbit anti-eIF2α (phosphor S51) antibody, rabbit anti-StAR antibody, rabbit anti-CYP11A1 antibody, rabbit anti-CYP17A1 antibody, rabbit antiGAPDH antibody, rabbit anti-β-actin antibody, goat anti-mouse IgG, HRP-linked antibody and goat anti-rabbit IgG, HRP-linked antibody (Cell Signaling Technology, Beverly, MA, USA), glyphosate, poly-L-lysine solution (sigma-aldrich, Santa Louis, MO, USA), goat serum blocking solution (ZSJB-BIO, Beijing, China), 4',6-diamidino-2-phenylindole (DAPI) (Beyotime, Shanghai, China). PBA was dissolved as a stock solution (0.1 M) in PBS and the final concentration was 10 μM. GSK2606414 was dissolved as a stock solution (2 mM) in DMSO and the final concentration was 0.5 μM.

Cell cultures and treatment
TM3 cells (purchased from Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Shanghai, China), a mouse Leydig cell line, were cultured in DMEM/F12 complete medium containing 5 % horse serum and 2.5 % FBS (Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Shanghai, China) at 37 °C in a humidified atmosphere with 5 % CO_2. For cell viability test, cells were seeded at a density of 5 x 10^3 cells/well in 96-well plates. For ELISA, cells were seeded in 24-well plates (4 x 10^4 cells/well) and the concentration of testosterone in cell culture medium was detected by ELISA. For western blotting analysis, cells were seeded at a density of 4 x 10^5 cells/dish in Ø 60 mm Petri dishes. Cells were cultured for 24 h before experimental treatment.

Cell viability test
Cell viability was tested using CCK-8 Kit according to the manufacturer's protocol. Briefly, TM3 cells were seeded in 96-well plates for 24 h before the treatment. After exposed to glyphosate at different concentrations from 0.01 to 2000 mg/L for 24 h, cells in each well were added with 10 μL reagent from the Cell Counting Kit and then incubated at 37 °C for 80 min. Finally, OD value of each well at the wavelength of 450 nm was read by a multimode plate reader (Thermo Scientific, NYC, NY, USA). The experiment was repeated independently four times, and six duplicate wells were set in each group.

Testosterone determination by ELISA
TM3 cells were cultured in a 24-well plate and exposed to glyphosate at different concentrations for 24 h with or without PBA or GSK2606414 pre-treatment. Then, the medium was collected and centrifuged at 12,000 rpm for 5 min at 4 °C. The testosterone in supernatant was assayed using ELISA kit according to the manufacturer's protocol. Briefly, each well of ELISA plate was added with 40 μL sample dilution, 10 μL sample and 100 μL enzyme labelling reagent respectively, then the plate was covered and incubated at 37 °C for 60 min. After thoroughly aspirating solution from wells, the plate was washed with washing solution for five times. Then each well was added with 50 μL developer A and 50 μL developer B, and incubated at 37 °C for 15 min in the dark. Finally, 50 μL stop solution was added to terminate the reaction. The absorbance of each well was measured at a wavelength of 450 nm. The experiment was repeated independently four times, and three duplicate wells were set in each group.

Western blotting analysis
Cells were seeded in Ø 60 mm Petri dishes. After exposed to glyphosate for 24 h with or without PBA or GSK2606414 pre-treatment, cells were extracted using the RIPA lysis buffer (Beyotime, Shanghai, China) containing 1 % phenylmethylsulfonyl fluoride (PMSF) and protease inhibitor cocktail. Protein concentrations were detected by BCA protein assay kit (Beyotime, Shanghai, China). Equal amount of protein was prepared and separated by 10 % SDS-PAGE. Then, protein was transferred to a nitrocellulose membrane (Whatman, Dassel, Germany). After blocking with 5 % bovine serum albumin in Tris buffered saline (TBS) for 2 h, the membranes were incubated with primary antibodies overnight at 4 °C. Next day, the membranes were washed 3 times with TBS containing 0.1 % tween-20 (TBST) for 10 min/time and incubated with secondary antibody for 1 h at room temperature. After washed with TBST, the bolts were detected with BeyoECL Star (Beyotime, Shanghai, China) and the grey scales of protein bands were measured by a Chemiluminescence Imager (BIO-RAD, Hercules, CA, USA). Experiments were repeated independently for eight times.

Immunofluorescence analysis
For immunofluorescence analysis, glass slides were coated with 0.01 % (w/v) poly-L-lysine solution for 5 min. The excess solution was removed and the slides were dried at room temperature. Then the slides were put into Ø 35 mm Petri dishes and cells were seeded in them. After exposed to glyphosate for 24 h with or without PBA or GSK2606414 pre-treatment, cells were fixed with 4 % paraformaldehyde for 20 min and permeabilized with 0.5 % Triton X-100 for 15 min at 4 °C. Then, cells were treated with goat serum blocking solution for 2 h at room temperature and incubated with primary antibodies overnight at 4 °C. Subsequently, cells were incubated with TRITC-conjugated secondary antibody for 1 h and DAPI for 15 min at room temperature. Finally, the stained slides were photographed with laser scanning confocal microscope (Olympus, Tokyo, Japan) and the fluorescence intensity was evaluated using ImageJ software. Each experiment was repeated four times and two background groups (without primary antibody or secondary antibody) were set.

Statistical analysis
Data were presented as mean ± SD and analysed by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls (SNK) test using SPSS 22 statistical software. A difference at $p < 0.05$ was considered statistically significant.

Results

Glyphosate at low concentration inhibited testosterone secretion but not to affect cell viability in TM3 cells
After treated with glyphosate at different concentrations from 0.01 to 2000 mg/L for 24 h, cell viability was measured using CCK8 and the concentration of testosterone in the culture medium was detected using ELISA kit. The results showed that glyphosate at and above 500 mg/L could decrease TM3 cell viability ($p < 0.05$), whereas glyphosate at concentrations at and below 200 mg/L had no significant effect on TM3 cell viability (Figure 1). In contrast to cell viability, as shown in Figure 2, exposure to glyphosate at and above a concentration above 0.5 mg/L could significantly decreased the concentrations of testosterone in culture medium ($p < 0.05$). These findings suggested that glyphosate at low concentrations, which had no effect on cell viability, could inhibit testosterone secretion in TM3 cells.

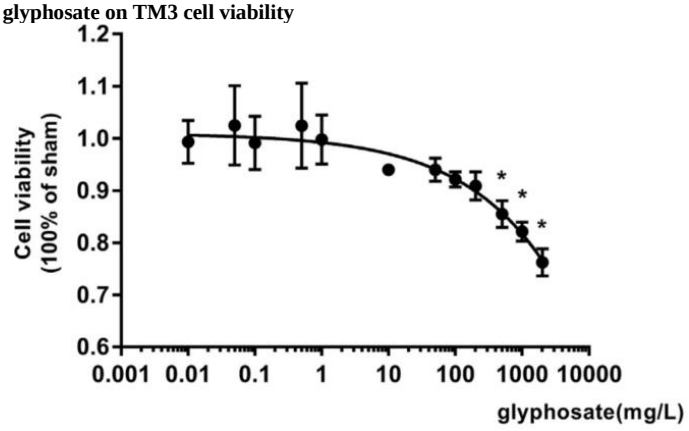
Glyphosate inhibited the expression of testosterone synthases StAR and CYP17A1
In the experiment, the effect of glyphosate on expression of testosterone key synthases was investigated. TM3 cells were treated with 5 mg/L glyphosate for different durations (1, 3, 6, 12 or 24 h), and the protein level of testosterone synthases was detected by western blotting. The results showed that glyphosate exposure significantly reduced the protein levels of StAR and CYP17A1 ($p < 0.05$) (Figure 3A and B). However, the protein levels of CYP11A1 and 3β-HSD were not affected by glyphosate (Figure 3C and D). It suggested that glyphosate decreased testosterone secretion through selectively inhibiting expression levels of key synthesis enzymes of StAR and CYP17A1 in TM3 cells.

Glyphosate induced ER stress and activated the PERK/eIF2α signaling pathway in TM3 cells
To investigate whether glyphosate exposure causes endoplasmic reticulum (ER) stress and activates the PERK/eIF2α signaling pathway in TM3 cells, the expression levels of related proteins were detected by western blotting. Results found that exposure to glyphosate at 5 mg/L for 1 h significantly increased all of the protein levels of Bip, p-PERK, PERK and p-eIF2α, which peaked at around 12 h ($p < 0.05$) (Figure 4A-D), but the protein level of eIF2α was not affected by glyphosate (Figure 4E). When TM3 cells were pretreated with 10 mM PBA, an inhibitor of ER stress for 2 h, all of the glyphosate-induced increases in protein levels of Bip, p-PERK, PERK and p-eIF2α were completely inhibited (Figure 5A-D) ($p < 0.05$). These results indicated that glyphosate could induce ER stress and the activation of PERK/eIF2α signaling pathway in TM3 cells.

Glyphosate-induced testosterone synthesis disorders depended on ER stress
In the present experiment, the role of ER stress in glyphosate-induced testosterone synthesis disorders was investigated. The results showed that PBA, the inhibitor of ER stress not only significantly rescued glyphosate-inhibited expressions both of StAR and CYP17A1 ($p < 0.05$), but also effectively restored the testosterone secretion inhibited by glyphosate ($p < 0.05$) (Figure 6). It indicated that glyphosate-induced testosterone synthesis disorders were mediated by the ER stress.

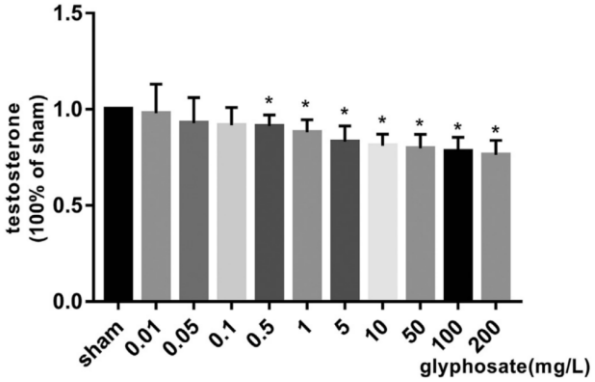
PERK/eIF2α signaling pathway mediated glyphosate-induced testosterone synthesis disorders
To investigate the role of PERK/eIF2α pathway in the glyphosate-induced testosterone synthesis disorders, TM3 cells were pretreated with 0.5 μM GSK2606414, an inhibitor of PERK activation for 2 h, and then incubated with 5 mg/L glyphosate for 24 h. The results showed that GSK2606414 pre-treatment significantly reduced glyphosate-induced phosphorylation of PERK and eIF2α ($p < 0.05$) (Figure 7). In addition, the decrease both of StAR and CYP17A1 protein expression induced by glyphosate was completely blocked ($p < 0.05$), and the glyphosate-inhibited testosterone secretion also was effectively rescued ($p < 0.05$). These results suggested glyphosate-induced testosterone synthesis disorders depended on StAR and CYP17A1 which mediated by PERK/eIF2α signaling pathway.

Figure 6.8.3.19-1: Effects of glyphosate on TM3 cell viability



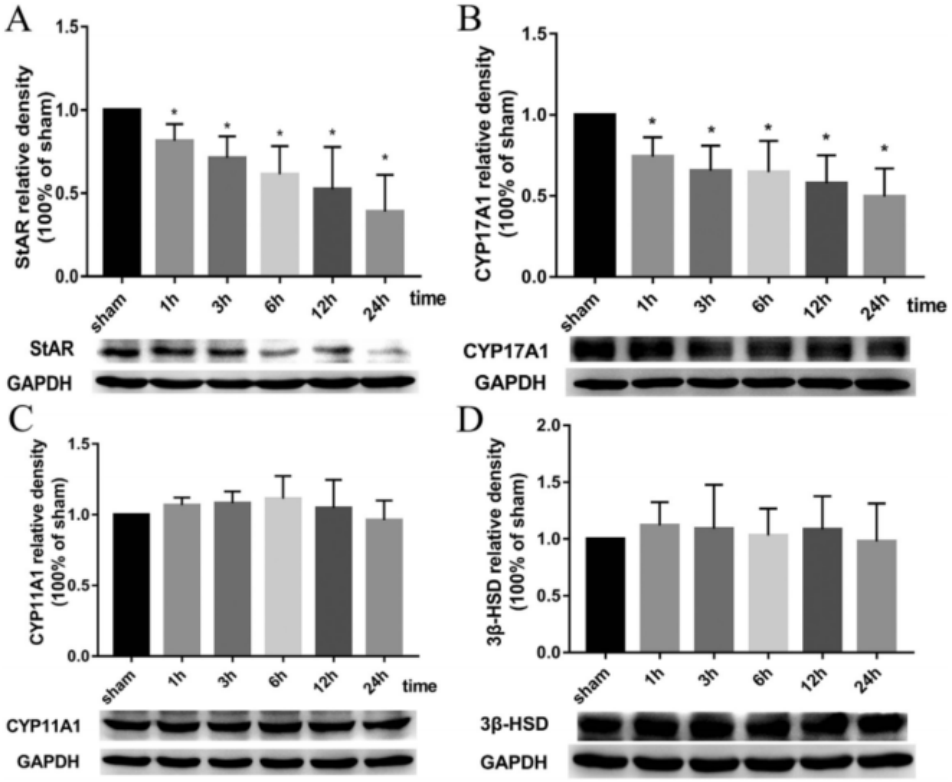
TM3 cells were exposed to different doses of glyphosate in 96-wells plate for 24 h and cell viability was measured using CCK8. Data were presented as the mean ± SD for n = 4 independent experiments.
*$p < 0.05$, compared with the sham group.

Figure 6.8.3.19-2: Glyphosate inhibited testosterone secretion of TM3 cells



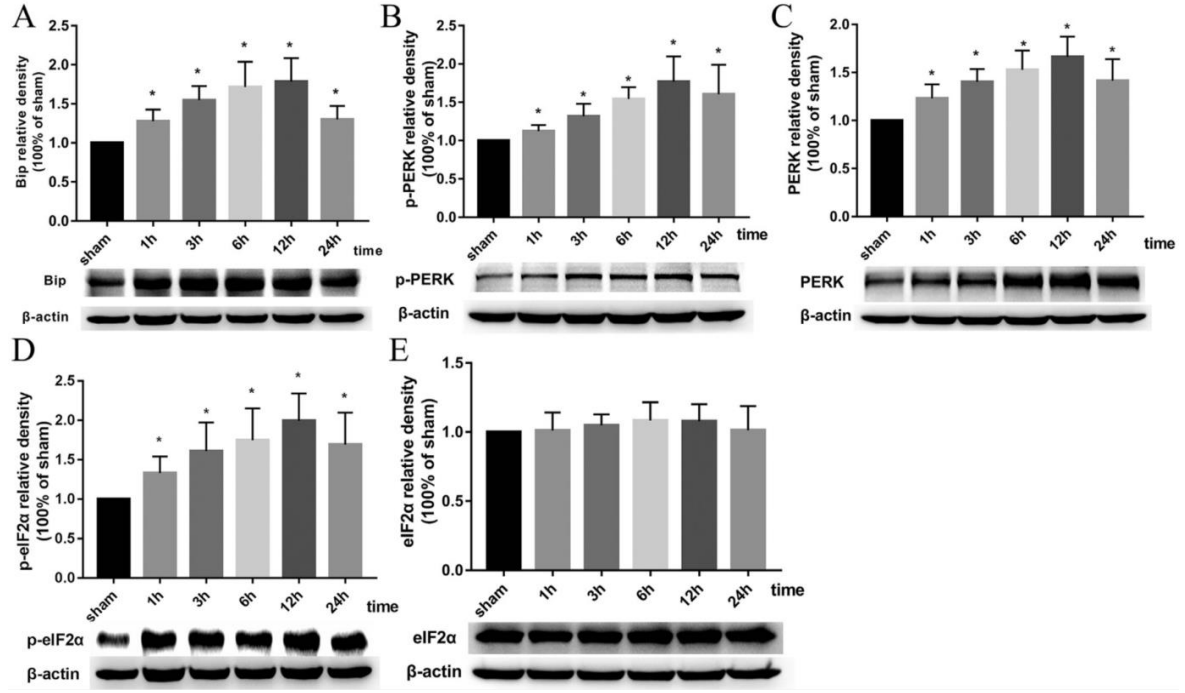
TM3 cells were exposed to different doses of glyphosate for 24 h. The cell supernatants were collected and the testosterone was assayed using ELISA kit. Data were presented as the mean ± SD for n = 4 independent experiments.
*p <0.05, compared with the sham group.

Figure 6.8.3.19-3: Glyphosate inhibited the protein expression levels of testosterone synthesis enzymes in TM3 cells



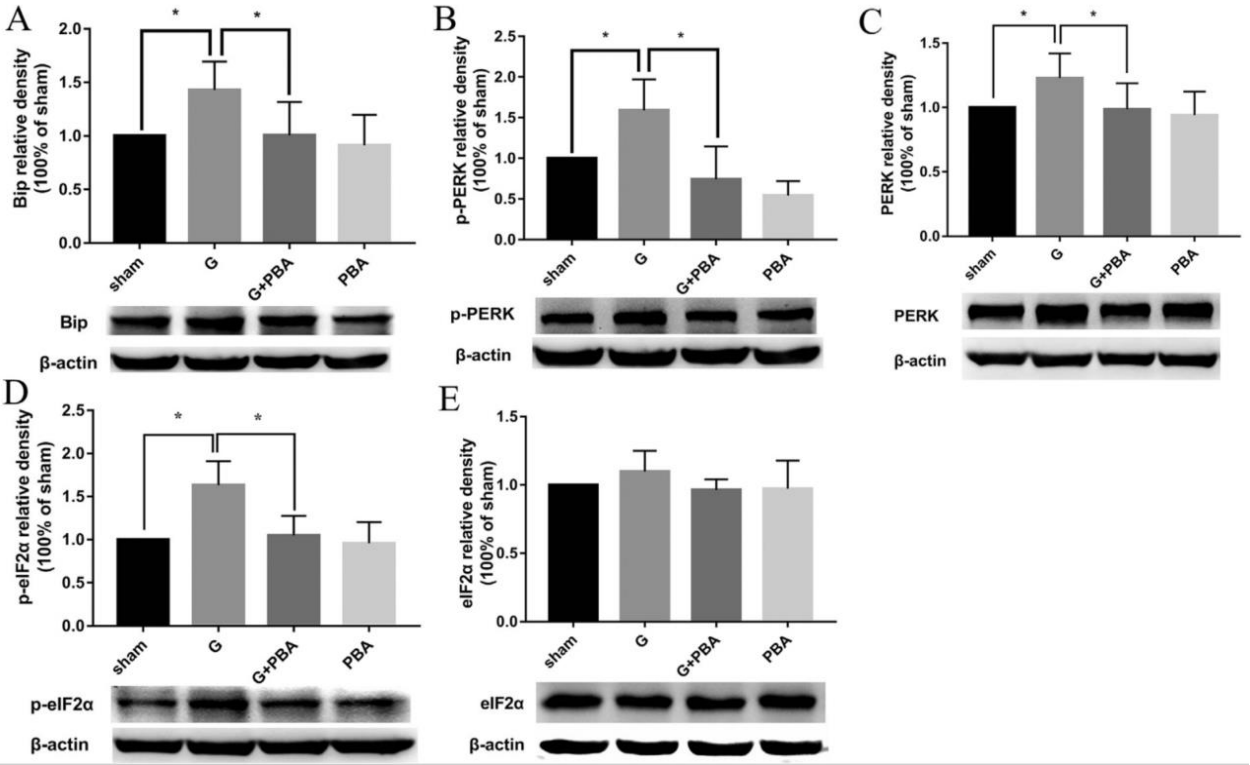
TM3 cells were exposed to 5 mg/L glyphosate for 1-24 h. The level of StAR, CYP17A1, CYP11A1 and 3β-HSD were detected with western blotting. Data were presented as the mean ± SD for n = 8 independent experiments.
*p <0.05, compared with the sham group.

Figure 6.8.3.19-4: Glyphosate induced ER stress and activated the PERK/eIF2α signalling pathway in TM3 cells



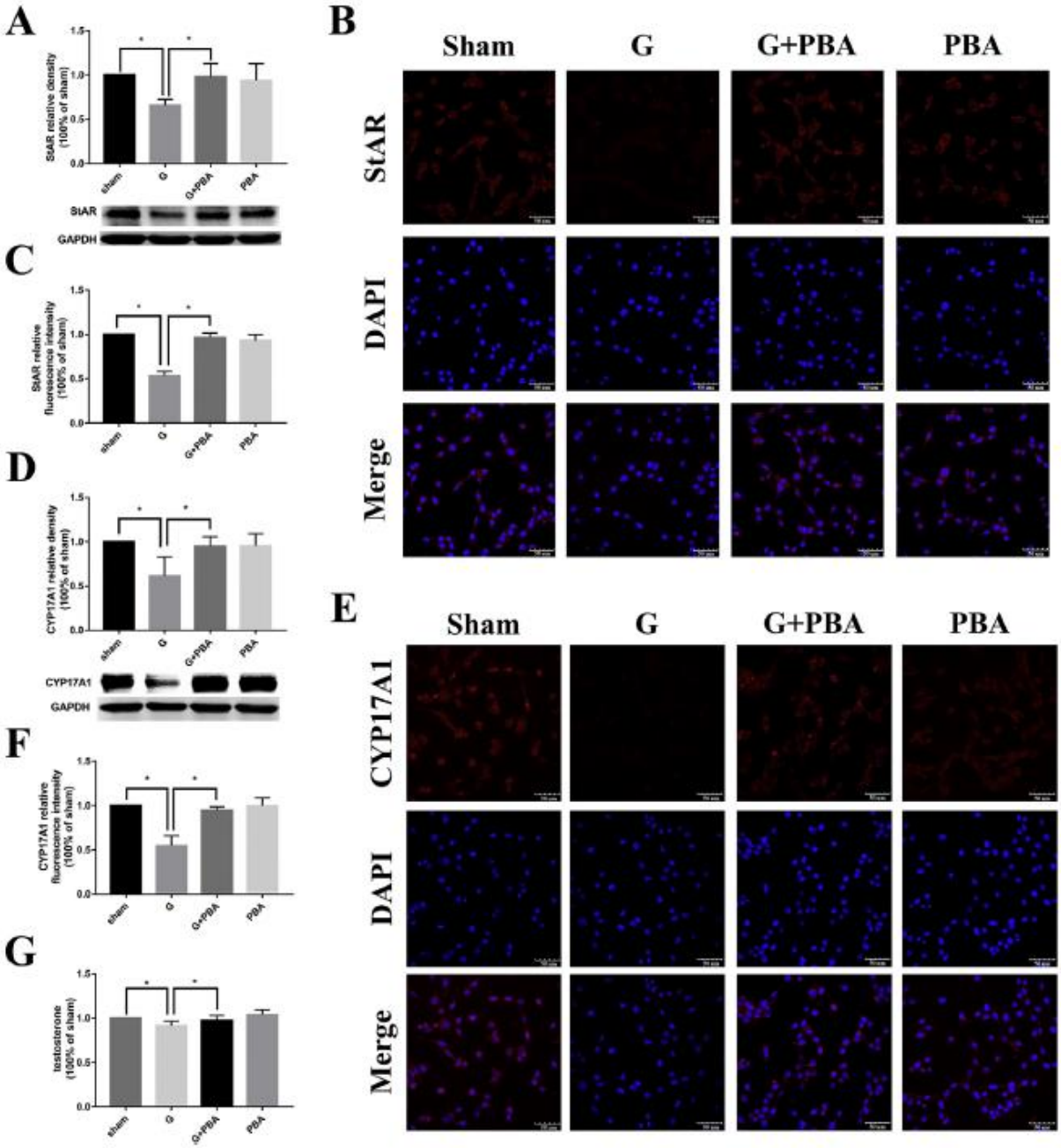
TM3 cells were exposed to 5 mg/L glyphosate for 1-24 h. The level of Bip, p-PERK, PERK, p-eIF2α and eIF2α were detected with western blotting. Data were presented as the mean ± SD for n = 8 independent experiments.
*p <0.05, compared with the sham group.

Figure 6.8.3.19-5: PBA alleviated ER stress induced by glyphosate in TM3 cells



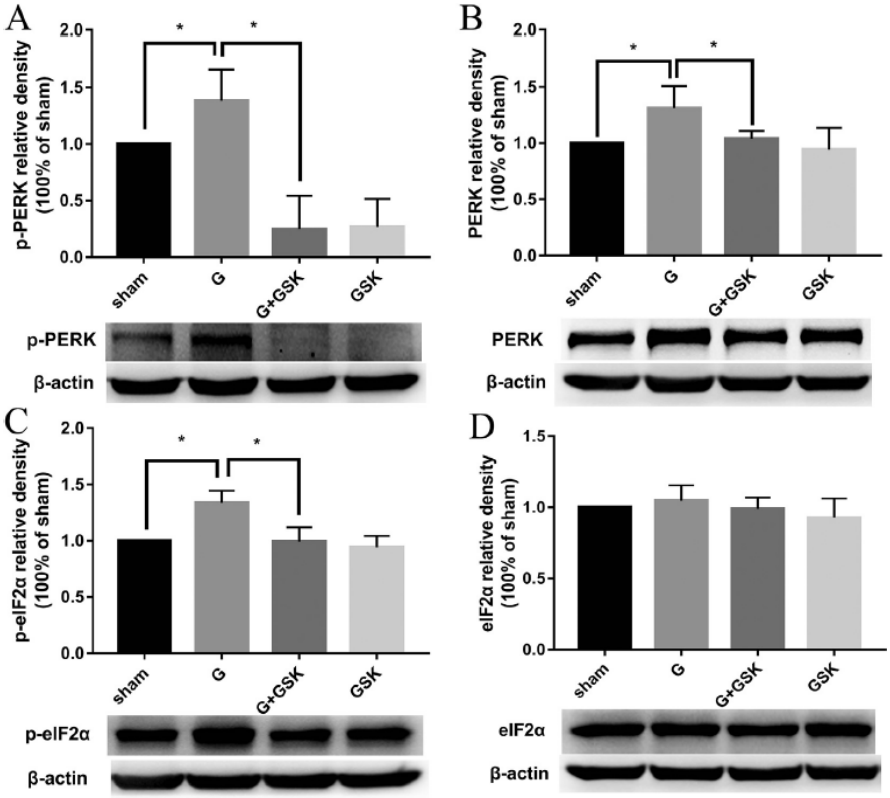
TM3 cells were pre-treated with 10 μM PBA for 2 h, and then incubated with 5 mg/L glyphosate for 24 h. The level of Bip, p-PERK, PERK, p-eIF2α and eIF2α were detected with western blotting. Data were presented as the mean ± SD for n = 8 independent experiments.
*p <0.05. Panel labels are Sham for negative control, G for glyphosate exposure only, G + PBA for PBA treatment before glyphosate exposure, and PBA for PBA treatment only. G: glyphosate; PBA: phenylbutyric acid.

Figure 6.8.3.19-6: PBA rescued the glyphosate-induced testosterone synthesis disorders.



TM3 cells were pretreated with 10 mM PBA for 2 h, and then incubated with 5 mg/L glyphosate for 24 h. A and D: The level of StAR and CYP17A1 were detected with western blotting. Data were presented as the mean ± SD for n = 8 independent experiments. B and C, E and F: Immunofluorescence stain was used to analyze the fluorescence intensity of StAR and CYP17A1. Data were presented as the mean ± SD for n = 4 independent experiments. G: The cell supernatants were collected and the testosterone was assayed using ELISA kit. Data were presented as the mean ± SD for n = 4 independent experiments. *$p < 0.05$. Panel labels are Sham for negative control, G for glyphosate exposure only, G+PBA for PBA treatment before glyphosate exposure, and PBA for PBA treatment only. G: glyphosate; PBA: phenylbutyric acid.

Figure 6.8.3.19-7: GSK2606414 blocked PERK/eIF2a signaling pathway activated by glyphosate in TM3 cells.



TM3 cells were pretreated with 0.5 mM GSK2606414 for 2 h, and then incubated with 5 mg/L glyphosate for 24 h. The levels of p-PERK, PERK, p-eIF2a and eIF2a were detected with western blotting. Data were presented as the mean ± SD for n = 8 independent experiments. *p < 0.05. Panel labels are Sham for negative control, G for glyphosate exposure only, G+GSK for GSK2606414 treatment before glyphosate exposure, and GSK for GSK2606414 treatment only. G: glyphosate; GSK: GSK2606414.

Conclusions

The authors conclude that glyphosate could inhibit testosterone synthesis via decreasing the expression of testosterone synthase StAR and CYP17A1, which depends on the ER stress and activation of PERK/eIF2α signalling pathway in Leydig cells.

Assessment and conclusion by applicant:

In vitro study on the effects of glyphosate on testosterone secretion and the role of endoplasmic reticulum stress in the process were investigated in TM3 cells. Results showed that exposure to glyphosate at concentrations below 200 mg/L had no effect on cell viability, while glyphosate at concentrations above 0.5 mg/L could inhibit the testosterone secretion in TM3 cells. Treatment of TM3 cells with glyphosate at 5 mg/L not only reduced the protein levels of testosterone synthase StAR and CYP17A1 but also inhibited testosterone secretion.

The article is classified as reliable with restrictions for the following reason: not enough information on the tested material (purity) was provided, no positive controls were used, and no statistical methods were described. Furthermore, no OECD guidelines were followed, no GLP status was stated, and no historical control data (HCD) were provided to compare the relevance of data. In addition, key literature in disagreement with the authors' findings appear to have been disregarded, suggesting bias within the research and the following publications: Hecker (2011), OECD validation of the H295R steroidogenesis assay with glyphosate; Levine (2007), demonstrating a lack of effect of glyphosate on the StAR protein; US EPA (2015), glyphosate EDSP weight of evidence evaluation; and EFSA (2017), peer review of glyphosate potential endocrine-disrupting properties.

Hecker M et al (2011), The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter-laboratory validation study, Environmental Science and Pollution Research 18(3):503-15

Levine S. L. Et al. (2007), Disrupting mitochondrial function with surfactants inhibits MA-10 Leydig cell steroidogenesis, Cell Biol Toxicol (2007) 23:385-400

US EPA (2015), EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals - EDSP: WEIGHT OF EVIDENCE ANALYSIS OF POTENTIAL INTERACTION WITH THE ESTROGEN, ANDROGEN OR THYROID PATHWAYS - CHEMICAL: GLYPHOSATE

EFSA (2017): Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate, Question number: EFSA-Q-2016-00663

Reliability Criteria by applicant: *In Vitro* Toxicology Studies		
Publication: Xia Y. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	Not stated
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate Purity not stated
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	N	TM3 cells (no purity stated)
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	Not necessary
Test concentrations in physiologically acceptable range (<1 mM)	N	0.01 to 200 mg/L

Reliability Criteria by applicant: *In Vitro* Toxicology Studies		
Publication: Xia Y. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive controls
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	Not enough information on the tested material (purity), no positive controls were used, no statistical methods were described. Furthermore, no OECD guideline followed, no GLP status was stated. In addition, no HCD were available in order to compare the relevance of the data. In addition, key literature in disagreement with the authors' findings appear to have been disregarded, suggesting bias within the research and publication: Hecker (2011) OECD validation of the H295R steroidogenesis assay with glyphosate; Levine (2007) demonstrating a lack of effect of glyphosate on the StAR protein; US EPA (2015) glyphosate EDSP weight of evidence evaluation; EFSA (2017) peer review of glyphosate potential endocrine-disrupting properties.
Not reliable	N	

Assessment and conclusion by RMS:
In this *in vitro* study, TM3 cells (murine Leydig cell line) were treated with glyphosate at concentrations ranging from 0.01-2000 mg/L for 24 h. Cell viability was significantly decreased ≥ 500 mg/L, whereas a significant decrease in testosterone secretion was observed at ≥ 0.5 mg/L. Additional experiments were performed at 5 mg/L glyphosate to investigate the testosterone secretion and the role of endoplasmic reticulum (ER) stress in this process. Treatment of TM3 cells with glyphosate at 5 mg/L reduced the testosterone secretion via a decrease in protein levels of the testosterone synthases StAR and CYP17A1, but not CYP11A1 and 3β-HSD. Furthermore, glyphosate induced ER stress in this study and activated the PERK/eIF2α signalling pathway in TM3 cells. This process, however, could be alleviated by pre-treatment with PBA, an inhibitor of ER stress. Lastly, following pre-treatment of TM3 cells with GSK2606414 (a PERK inhibitor), testosterone secretion was not inhibited. Overall, the authors concluded that glyphosate can interfere with the expression of StAR and CYP17A1 and inhibit testosterone synthesis and secretion via ER stress-mediated the activation of PERK/eIF2a signalling pathway in Leydig cells.

The applicant lists a few reasons why the study should be considered reliable with restrictions (see above). While the RMS agrees and further notes that the negative control (sham) is unknown. Based on the available data, it cannot be ascertained that the effects observed in the experiments are glyphosate-related or whether the unknown negative control is responsible for these effects. In conclusion, the study is therefore considered to be reliable with restrictions (Klimisch score 2).

B.6.8.3.20. Public literature – Analysis endocrine disruption effect of Roundup in adrenal gland male rats (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.8.3
Report author	Pandey A. *et al.*
Report year	2015
Report title	Analysis of endocrine disruption effect of Roundup in adrenal gland of male rats
Document source	Toxicology Reports (2015), Vol. 2, pp. 1075-1085. Supplementary material related to the article is available at http://dx.doi.org/10.1016/j.toxrep.2015.07.021
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

The publication aims at the investigation of the effect of Roundup procured in India (batch and purity not reported) on adrenal gland steroidogenesis and signalling pathway associated with steroid production. For a period of 14 days, groups of 5-10 male rats were administered to the vehicle (water) or dose levels of 4.4, 121.9, 244 or 609.8 µL Roundup (41% w/w), corresponding to 10, 50, 100 and 250 mg/kg bw/day of glyphosate, respectively.

The animals were observed for clinical signs of toxicity and body weight and food consumption were recorded for each animal over the whole study period. Upon treatment with Roundup, circulatory levels of steroids and the expression of genes associated with steroidogenesis was investigated. Further, the circulatory and adrenal gland lipid and cholesterol content was investigated and the expression of genes involved in cholesterol intake and *de novo* synthesis was monitored. In addition, the effect of Roundup on esterified cholesterol (CE) and ester

hydrolase was investigated and adrenal gland ACTH receptor expression and circulatory ACTH levels were analysed after treatment with Roundup.

To examine the effect of exogenous adrenocorticotropic hormone (ACTH) on adrenal gland steroidogenesis, two additional groups of 3 rats treated with the vehicle or 10 mg/kg bw/day glyphosate for 14 days were injected with 5 IU of porcine ACTH intravenous one hour prior to sacrifice. Afterwards, the effect of exogenous ACTH upon circulatory corticosterone levels and the effect on adrenal gland PKA activity were examined in Roundup treated animals. In addition, the effect of Roundup on cell toxicity was investigated.

There was a decrease in body weight and a decrease in food consumption at 50 mg/kg bw/day and above, yielding statistical significance at ≥100 mg/kg bw/day. In addition, a statistically significant decrease in circulatory corticosterone at ≥10 mg/kg bw/day and a statistically significant decrease in testosterone at 50 mg/kg bw/day was observed. The 10 mg/kg bw/day dose which reduced circulatory corticosterone levels, but did not change food consumption and body weight, was selected for further study.

The key regulatory steps in steroidogenesis are transport of cholesterol from outer to inner mitochondrial membrane by StAR protein and cholesterol side-chain cleavage step by P450scc enzyme. After treatment with 10 mg/kg bw/day glyphosate/ Roundup, StAR mRNA and total protein were statistically significantly decreased, when compared to the vehicle group. The phosphorylated form of CREB, the transcriptional regulator of StAR expression, and the phosphorylated StAR protein were also found to be statistically significantly decreased.

There was no change in circulatory total, free and esterified cholesterol in Roundup-treated rats, but a dose-dependent increase in high- and low- density lipoprotein (HDL and LDL) levels. The expression of cholesterol receptor (LDL receptor), the de novo cholesterol synthesis enzyme (3-hydroxy-3-methylglutaryl-coenzyme A synthase) and hormone-sensitive lipase were significantly decreased when compared to control animals.

There was no change in expression of the adrenocorticotropic hormone receptor (ACTH) melanocortin-2 (Mc2r), but circulatory ACTH levels and adrenal cortex protein kinase A (PKA) activity were statistically significantly reduced. Surprisingly, exogenous ACTH treatment rescued steroidogenesis in Roundup-treated animals. Apoptosis was evident at 250 mg/kg bw/day, but not at 10 mg/kg bw/day dose. These results suggest that Roundup may be inhibitory to hypothalamic-pituitary axis leading to reduction in cyclic adenosine monophosphate (cAMP)/PKA pathway, StAR phosphorylation and corticosterone synthesis in the adrenal tissue.

Materials and methods

Test Material:	Glyphosate formulation Roundup, 41% (w/w)
Origin:	Monsanto India Ltd., Mumbai, India
Lot/Batch number:	Not reported
Purity:	Not reported
CAS#:	Not reported
Vehicle:	Water
Test Animals:	
Species	Rat
Strain	Wistar
Sex:	Male
Age at treatment	2-2.5 months
Source	Harlan
Housing	Rats were caged individually.
Diet	Standard chow diet, *ad libitum*
Water	*ad libitum*
Environmental conditions	Temperature: 24-26 °C Photoperiod: 12 hours light / 12 hours dark

Chemicals and antibodies
Porcine ACTH, TRIzol, custom made primers, oligo(dT) and dNTPs were obtained from Sigma-Aldrich Co. (Bangalore, India). The kits purchased for various hormone assays were as follows: rat corticosterone ELISA

from Neogen (Lansing, MI), rat corticosterone EIA from Cayman (Ann Arbor, MI), testosterone RIA from Immunotech (Marseille, France) and rat ACTH Ultra-sensitive lumELISA kit from Calbiotech Inc (Spring Valley, CA). Amplex red cholesterol assay kit and SYBR Green PCR Master Mix were purchased from Molecular Probes, Life Technologies (Carlsbad, CA). Reverse transcriptase (RevertAid) was from Thermo Scientific (Waltham, MA). DNase 1 (RNase free) was from New England Biolabs Inc. (Ipswich, MA). PVDF membrane (Immobilon pSQ) was procured from Millipore (Billerica, MA). Protein molecular weight markers (PageRulerTM Prestained Protein Ladder) and Western blotting detection reagents (SuperSignalTM West Femto Maximum Sensitivity Substrate) were from Thermo Fisher Scientific Inc. (Waltham, MA). Antibody against pCREB (Ser133) (#9198), CREB (#9197), cleaved Caspase 3 (#9915), goat anti-rabbit IgG, HRP-linked Antibody (#7074) were from Cell Signaling Technology (Danvers, MA) and -actin (#A3854) from Sigma-Aldrich Co. (Bangalore, India). Antibodies for pStAR and StAR were kind gifts from Professor Steven King (Baylor College of Medicine, Houston, TX) and Professor DM Stocco (Texas Tech University Health Sciences Center, Lubbock, TX). HDL and LDL/VLDL quantitation kit was procured from Sigma-Aldrich Co. (Bangalore, India). All other chemicals, unless otherwise noted were purchased from Sigma-Aldrich Co. (Bangalore, India) or sourced locally.

Animal assignment and treatment

The test item was dissolved in water and administered to groups of 5-10 rats/dose daily by oral gavage for a period of 14 days (Table 1). The animals received doses of 4.4, 121.9, 244 or 609.8 µL Roundup (41% w/w), corresponding to 10, 50, 100 and 250 mg/kg bw/day of glyphosate, respectively. Dose levels were selected based on the LD_{50}, with the highest dose level being well below the LD_{50} of 4900 mg/kg bw/day. A constant dose volume of 300 µL was chosen, with the exception of the highest dose of 250 mg/kg bw/day being administered directly to the animals. A group of control animals received the vehicle (water).

To examine the effect of exogenous adrenocorticotropic hormone (ACTH) on adrenal gland steroidogenesis, two additional groups of 3 rats treated with the vehicle or 10 mg/kg bw/day glyphosate for 14 days were injected with 5 IU of porcine ACTH intravenous one hour prior to sacrifice.

Table 1. Animal assignment for the treatment with glyphosate (Roundup)

Dose level (mg/kg bw/day)	Number of animals
0	10
10	10
50	5
100	10
250	10
0 + 5 IU ACTH	4
10 + 5 IU ACTH	3

ACTH: adrenocorticotropic hormone

Observations

Body weight and food consumption were recorded twice weekly. Food consumption was calculated by subtracting food pellet weight remaining in the cage mesh from the total food pellet weight provided to each rat cage mesh. After 14 days of treatment, the final body weight was recorded and blood was collected by cardiac puncture. Plasma samples were generated for the hormone assay.

Pathology procedures

At scheduled necropsy on study Day 15, the animals of the main group were anaesthetized with 50 mg/kg bw pentobarbitone sodium and killed by cervical dislocation. Adrenal glands were dissected out, weighed and transferred to neutral buffered formaldehyde solution or snap-frozen in liquid nitrogen and stored in-70 ◦C freezer until analysis.

Animals for the ACTH assay received an intravenous dose of 5 IU ACTH and blood samples and adrenal glands were collected 60 minutes after ACTH treatment.

Hormone assays

Plasma corticosterone levels were determined with two different kits. The steroids extraction from plasma was carried out by using diethyl ether or methylene chloride (Merck, Billerica, MA) as per requirements of the kits. The corticosterone levels obtained from different sources gave a similar concentration of corticosterone. The

inter- and intra-coefficient of variations of the assay were <15%. Plasma concentrations of testosterone were measured by testosterone RIA kit according to the manufacturer's protocol. ACTH in plasma was measured using a luminescence-based ELISA kit for rats.

Cholesterol assay

Adrenal gland tissue lysate was prepared by homogenizing 0.5 mg tissue in unit mL of 10% SDS containing phosphate-buffered saline (PBS) (Sigma-Aldrich Co., Bangalore, India). Tissue debris was removed by centrifugation. The tissue lysate and plasma were analyzed for total cholesterol and esterified cholesterol by using the Amplex red cholesterol assay kit as per the manufacturer's instructions. Briefly, plasma or tissue sample was mixed with an equal volume of Amplex red working reagent with and without cholesterol esterase. The reaction mixture was then incubated for 30 min at 37 ◦C in the dark. The fluorescence values were read at an excitation wavelength of 545 nm and an emission wavelength of 590 nm (Tecan Infinite F200 Microplate Reader, Männedorf, Switzerland). A series of cholesterol standards were prepared that were provided in the kit and ran alongside the plasma and tissue lysate samples. Plasma HDL (high-density lipoprotein) and LDL (low-density lipoprotein) were analyzed by commercially available HDL and LDL/VLDL quantitation kit according to the manufacturer's instruction.

qPCR analysis

mRNA expression of key regulatory receptors, enzymes and carrier proteins involved in the cholesterol homeostasis and steroidogenesis were determined by qPCR analysis using ABI 7500 Real-Time PCR instrument. Briefly, total RNA was isolated from adrenal glands by Trizol method, treated with DNaseI before performing reverse transcription for cDNA preparation with oligo(dT). Real-time PCR was performed with each reaction carrying 10 ng of cDNA. Primers have been preferably designed from exon junction sequences, except for gene. The details of primers employed along with the sequence source are provided in Table 2. The expression level of the individual gene was normalized to Rpl19 expression, which was used as a calibrator (internal control) for each cDNA sample. PCR for each sample was set up in duplicates and the average Ct value was used in the Ct equation.

Table 2. List of primers used for qPCR analysis of key regulatory receptors, enzymes and carrier proteins involved in the cholesterol homeostasis and steroidogenesis after treatment of rats with glyphosate (Roundup)

Gene	NCBI gene ID	Forward primer (5′3′)	Reverse primer (3′5′)
Rpl19	81767	CGTCCTCCGCTGTGGTAAA	AGTACCCTTCCTCTTCCCTATGC
Srb1	25073	TGGGATGAACGACTCGAGT	AGTACCATTGATCATGTTGCAC
Ldlr	300438	GAGTCCCCTGAGACATGCAT	GGGAGCAGTCTAGTTCATCCG
Hmgcr	25675	GGGTCAAGATGATCATGTCT	ATTCTCTTGGACACATCTTCAG
Hmgcs	29637	ACGATACGCTTTGGTAGTTG	AAGCCCTCGGTCAAAAAT
Hsl	25330	CCTGCAACAGAGACACTGC	CTCTGAGTTGCCCTTAAAGCTC
P_{450SCC}	29680	ACCCAACTCGTTGGTTGGA	CACGTTGATGAGGAAGATGGT
StAR	25557	GGCCCCGAGACTTCGTAA	TGGCAGCCACCCCTTGA
Mc2r	282839	GTCCCCCGTGTACTTTTTCATC	GGACGAACATGCAGTCAATGAT

Hematoxylin and eosin (H&E) staining

mRNA expression of key regulatory receptors, enzymes and carrier proteins involved in the cholesterol homeostasis and steroidogenesis were determined by qPCR analysis using ABI 7500 Real-Time PCR instrument. Briefly, total RNA was isolated from adrenal glands by Trizol method, treated with DNaseI before performing reverse transcription for cDNA preparation with oligo(dT). Real-time PCR was performed with each reaction carrying 10 ng of cDNA. Primers have been preferably designed from exon junction sequences, except for gene. The details of primers employed along with the sequence source are provided in the table above. The expression level of individual gene was normalized to Rpl19 expression which was used as a calibrator (internal control) for each cDNA sample. PCR for each sample was set up in duplicates and the average Ct value was used in the Ct equation.

Oil Red O staining

The 5 m thick cryosections of frozen adrenal glands were prepared and fixed in NBF, washed with PBS (Sigma-Aldrich Co., Bangalore, India) and stained with Oil Red O (ORO) (Sigma-Aldrich Co., Bangalore, India) in 60% isopropanol (Sigma-Aldrich Co., Bangalore, India) for lipid detection. For nuclear staining, sections were equilibrated in McIlvaine's Citric acid; Na2HPO4 buffer and stained with DAPI and the sections were visualized under fluorescent microscope (Olympus IX81 inverted microscope, Tokyo, Japan).

Immunoblot analysis

The adrenal gland tissue was homogenized using RIPA buffer with protease inhibitors and the lysates were stored at -70 ◦C until further use. Total protein estimation was performed by Bradford method (Bio Rad lab. Inc, Berkeley, CA). Tissue lysates (30 g protein) were resolved on 12% SDS PAGE and transferred onto PVDF membrane using a wet transfer unit (Bio Rad Laboratories, Berkeley, CA). Non-specific sites on the membrane were blocked using 10% milk in TBST (20 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.1% Tween 20) by incubating it for 1 h at room temperature. The membrane was incubated overnight at 4 ◦C with primary antibody at 1:1000 dilution specific for pCREB (Ser133), CREB, Cleaved Caspase-3, pStAR, StAR and -actin. The membrane was washed with TBST and incubated with secondary antibody (horseradish peroxidase-labelled anti-rabbit IgG) at 1:3000 dilutions. The bands were visualized using Western blot imaging system (Flourchem FC2, Cell Biosciences Inc., Santa Clara, CA) and the band intensity was quantitated by Gene tool software (Syngene, Cambridge, UK).

PKA assay
PKA assays were performed using SignaTECT cAMP-dependent protein kinase (PKA) assay kit (Promega, Madison, Wisconsin). The activity of PKA was determined by measuring the incorporation of 32P from [32P] ATP via adrenal gland PKA to biotinylated kemptide, a highly specific peptide substrate. Briefly, the medullary region of adrenal gland was removed carefully from the decapitated gland under dissecting microscope in ice-cold PBS solution. The adrenal medulla region-specific gene, PNMT expression was found to be undetectable in the dissected cortical fraction (data not shown). The cortical fraction was homogenized in cold extraction buffer containing 25 mM Tris-HCl, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM ME, 1 g/ml Aprotinin and 1 g/ml Leupeptin and centrifuged at 4 ◦C, 14,000 × g for 5 min. After protein estimation, 10 g of protein was used to perform the assay by incubating with [32P] ATP at 30 ◦C for 5 min. The reaction was terminated by adding stopping buffer provided with the kit and 10 l of reaction mixture was spotted onto SAM biotin capture membrane. The individual membrane square was dried and then transferred to liquid scintillation vials for counting in counter (Tri Carb B2910TR liquid scintillation analyzer, Waltham, MA). A control reaction without the substrate was also performed for determining the background activity which was subtracted from the total activity of samples.

TUNEL assay
Assay was performed using TACS TdT DAB in situ apoptosis detection kit (Trevigen Inc., Gaithersburg, MD). Briefly, NBF fixed adrenal gland from vehicle, 10 and 250 mg/kg bw/d treated animals was sectioned into 5 m thickness. Sections were cleared in xylene and rehydrated using ascending grades of alcohol solutions and PBS. Sections were treated with Proteinase K followed by TdT labelling reaction mix and buffer incubation. The reaction was stopped by TdT Stop Buffer provided with the kit. The sections were washed thoroughly in PBS and treated with HRP conjugated streptavidin in a humid chamber. After PBS washing, the sections were exposed to DAB provided in the kit, till development of brown colour, followed by haematoxylin staining for nuclei and the sections were observed under light microscope. The positive and negative controls for the technique were included by addition of nuclease during incubation and removal TdT enzyme from the labelling mix, respectively.

Statistical analysis - Data were expressed as mean ± SEM. A t-test was used to calculate p value between two groups. Multiple comparisons were made between vehicle and Roundup treatment groups using Bonferroni's test after one way ANOVA. Prism version 5 (GraphPad, California) was utilized for statistical analysis. A p-value <0.05 was considered statistically significant throughout.

Results

Effect of Roundup on body weight and food consumption
At 250 mg/kg bw/day, the animals showed no overt signs of toxicity during the whole treatment period. Body weight and food consumption were reduced at 50 mg/kg bw/day (statistically not significant) and statistically significantly decreased at 100 and 250 mg/kg bw/day. The effects were explained by Roundup-induced toxicity rather than endocrine-disrupting effects. Hence, doses higher than 50 mg/kg bw/day were not included for further analysis (Table 3).

Table 3. Average food consumption (g) per animal per day (A) and average body weight (g) during treatment with Roundup

(A)

Days of treatment	Control ($n=4$)	10 mg/kg bw/d ($n=4$)	50 mg/kg bw/d ($n=3$)	100 mg/kg bw/d ($n=3$)	250 mg/kg bw/d ($n=3$)
0–3	16.75 ± 1.2	16.3 ± 1.5	16.7 ± 0.4	16.1 ± 1.3	14.3 ± 2.1
4–7	21.6 ± 1.8	19.9 ± 1.6	18.55 ± 0.12	15.2 ± 1.04*	13.03 ± 0.8***
8–11	22.4 ± 1.9	20.2 ± 1.8	19.15 ± 0.6	13.5 ± 1.08***	11.8 ± 0.4***
12–15	23.19 ± 1.9	20.3 ± 1.7	18.4 ± 0.8	12.8 ± 1.1***	11.7 ± 0.4***

(B)

Days of treatment	Control ($n=6$)	10 mg/kg bw/d ($n=6$)	50 mg/kg bw/d ($n=3$)	100 mg/kg bw/d ($n=5$)	250 mg/kg bw/d ($n=5$)
0	233 ± 15.6	225.2 ± 15.1	192 ± 5.9	188.4 ± 7.1	187.6 ± 4.1
3	244.8 ± 14.4	235.2 ± 16.6	196.6 ± 4.4	178.4 ± 7.2**	173 ± 6.02**
7	253.5 ± 15.3	243.2 ± 17.2	201.3 ± 4.7	176.8 ± 8.3***	176.2 ± 11.6***
11	262.3 ± 17.04	248.2 ± 18.2	205.2 ± 7.2	178.8 ± 4.7***	160.4 ± 9.5***
15	270.8 ± 18.2	254.4 ± 20.5	207.5 ± 8.9*	170.4 ± 6.8***	176.6 ± 5.9***

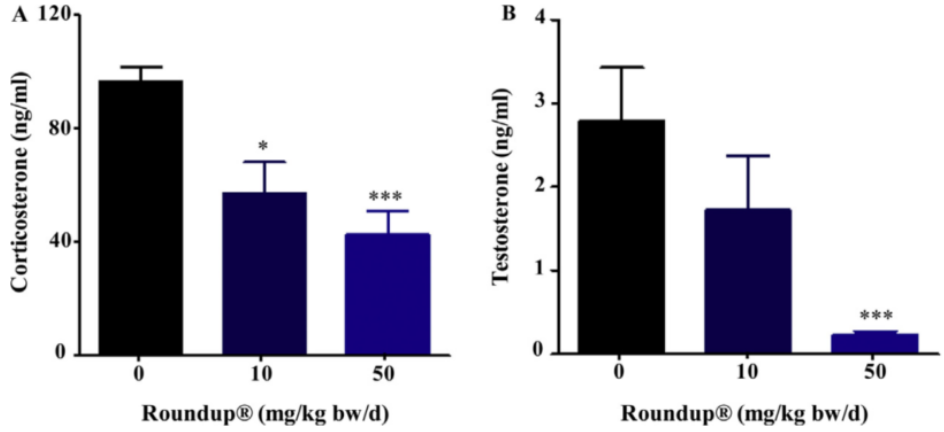
Values are mean ± SEM. Vehicle group received milliQ water for 14 days and other groups administered with different doses of Roundup® for 14 days. Statistical significance from vehicle group was determined by two way ANOVA followed by Bonferroni test.

* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

Effect of Roundup on circulatory levels of steroid

As representative steroid hormone from major endocrine sources, i.e. testis and adrenal gland of male rat, plasma testosterone and corticosterone levels were monitored. Both hormone concentrations were statistically significantly decreased after Roundup treatment in a dose-dependent manner. The lowest dose 10 mg/kg bw/d itself significantly decrease corticosterone levels ($p<0.05$), therefore this dose was selected for detailed endocrine-disrupting studies (Fig. 1).

Figure 1. Circulatory hormone levels in the vehicle and Roundup-treated animals after oral treatment for 14 days. Circulatory corticosterone (A) and testosterone (B) levels were measured at the end of 14 days of treatment. Data are presented as mean ± SEM (n = 5 per group). 't' test was performed to compare each treatment group to the vehicle group. *, ***, significantly different from vehicle group by $p<0.05$, 0.0001 viz.



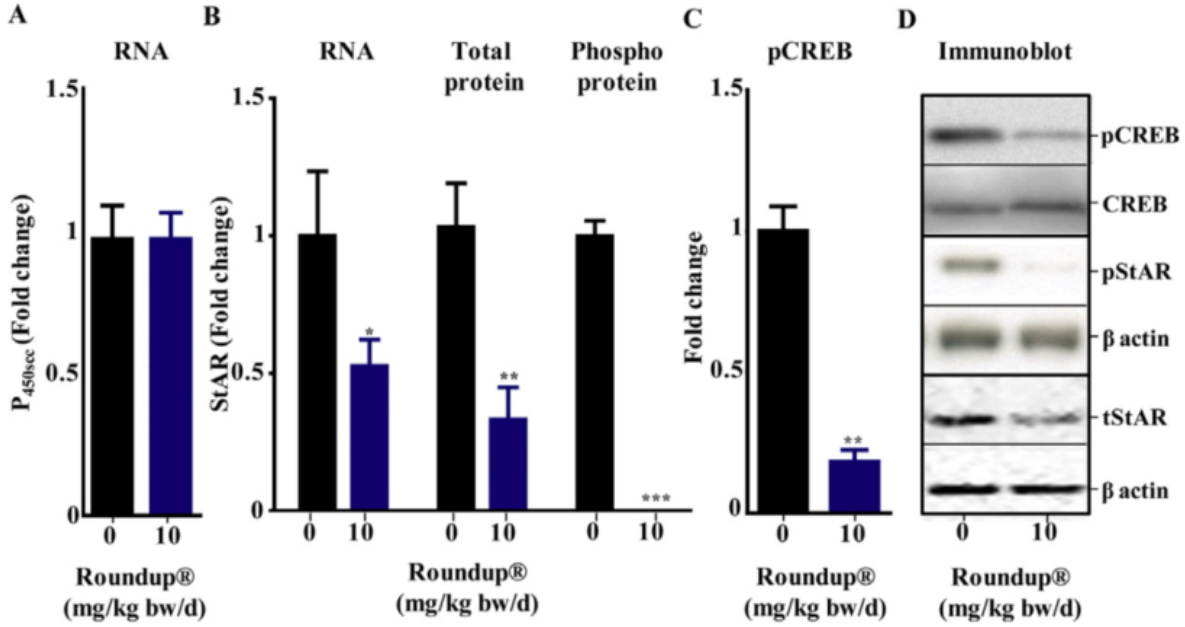
Effect of Roundup on expression of genes associated with steroidogenesis

The key regulatory steps in steroidogenesis are transport of cholesterol from outer to inner mitochondrial membrane by StAR protein and cholesterol side-chain cleavage step by P450scc enzyme. After treatment with 10 mg/kg bw/day glyphosate/ Roundup, the mRNA expression of P450scc was unchanged, while StAR mRNA and total protein were statistically significantly decreased in Roundup treated animals when compared to vehicle group. Phosphorylated form of CREB, the transcriptional regulator of StAR expression, was downregulated in Roundup-treated rats and phosphorylated StAR protein was also found to be significantly lower ($p<0.05$) in the 10 mg/kg bw/day treatment group.

The difference between total StAR and phosphorylated StAR expression levels post Roundup treatment, suggests gene regulation at two levels; one at transcription and another at phosphorylation process. Significant downregulation in phosphorylated StAR levels observed in the present study suggests decreased pStAR could be responsible for downregulation of steroidogenesis in the adrenal gland of Roundup-treated rats (Fig. 2).

The finding that phosphorylated CREB was lower may be correlated to StAR expression downregulation, since CREB phosphorylation is involved in StAR transcription.

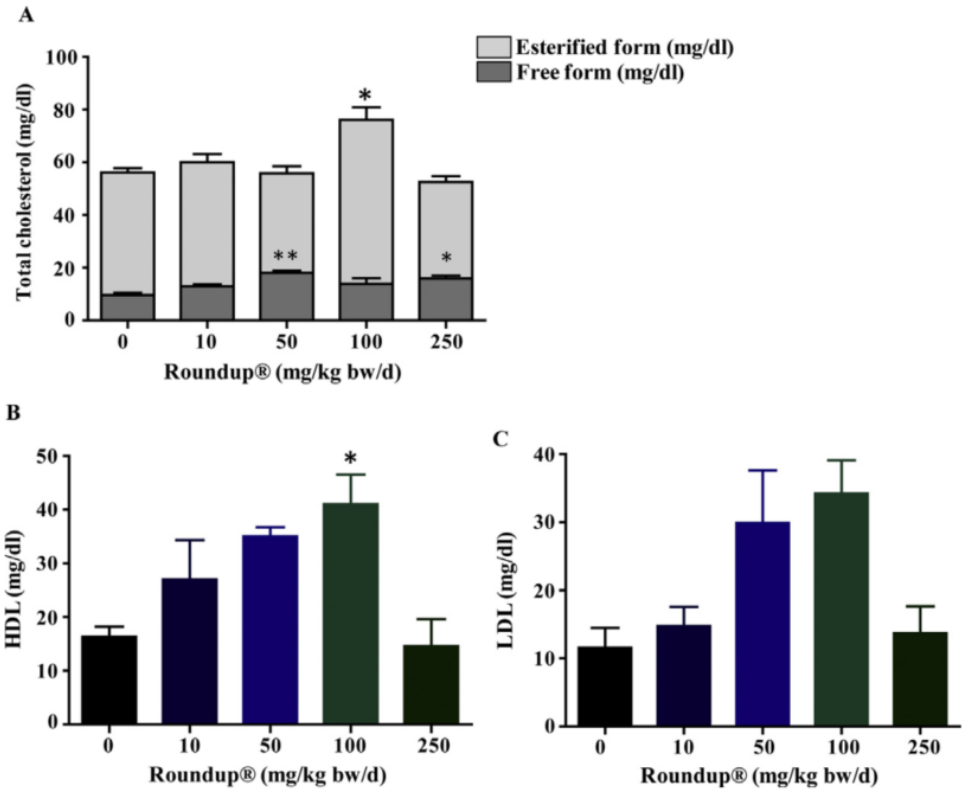
Figure 2. Expression of steroidogenic genes in rats treated with vehicle and 10 mg/kg bw/day Roundup for 14 days. Total RNA from adrenal gland was isolated and qPCR analysis was performed to quantitate the fold change of P450scc (A) and StAR gene expression (B). Rpl19 was used as internal control. The mRNA expression value in vehicle-treated animals was set as 1 fold and the mRNA expression value of the treated group was expressed in relation to the vehicle group. Immunoblot analysis of total and phosphorylated form of StAR and CREB proteins (C and D) was performed using the adrenal gland protein lysate from vehicle and Roundup-treated rats. Immunoblot densitometry arbitrary values for vehicle group was set as fold 1 and the values in treated groups were represented compare in comparison to the vehicle group. Blots are representative of three or more experiments. Values are presented as mean ± SEM (n = 5 per group). 't' test was performed to compare each treatment group with vehicle group. *, **, ***, significantly different from vehicle group by p <0.05, 0.01, 0.001.



Effect of Roundup on circulatory and adrenal gland lipid and cholesterol content

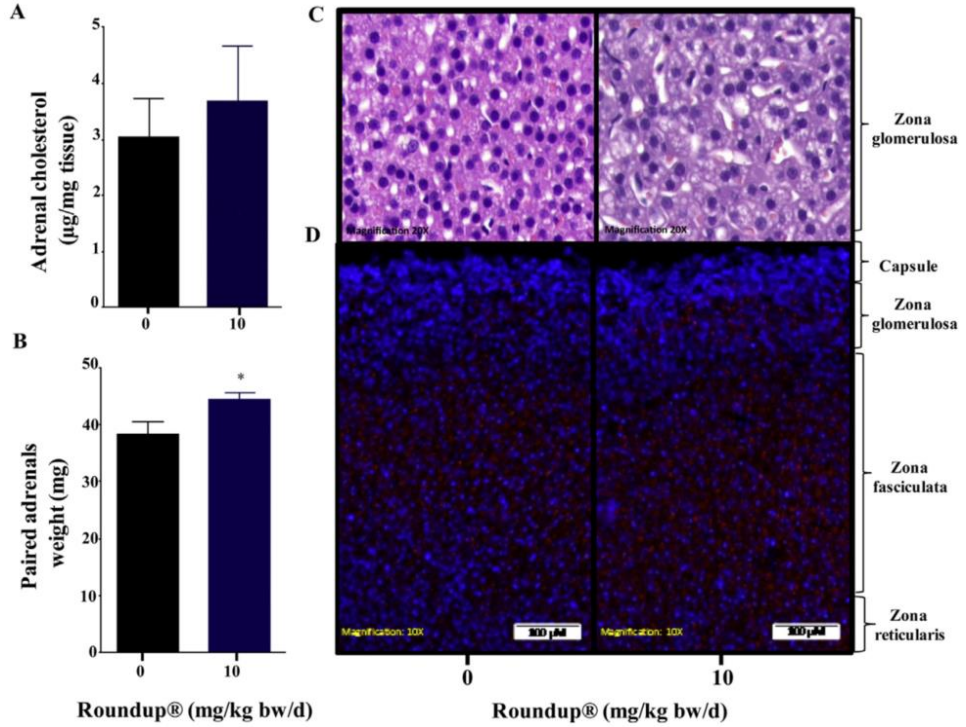
In addition to steroidogenesis, the homeostasis of steroid precursor cholesterol was examined in the circulation, as well as in the adrenal gland. There was no change in circulatory levels of total, free and esterified cholesterol after treatment with Roundup, except for the 100 mg/kg bw/day dose. In contrast, there was a dose-dependent increase in HDL and LDL levels observed, with a statistical significance at 100 mg/kg bw/day for HDL levels (Fig. 3).

Figure 3. Plasma cholesterol and lipoprotein (HDL and LDL) levels in vehicle- and Roundup-treated animals. Values are presented as mean ± SEM (n = 3-4 per group). 't' test was performed to compare each treatment group with the vehicle group. *,**, p <0.05, p <0.01 viz.



There was a slight increase in cholesterol levels (statistically not significant) found in the adrenal gland. The weight of adrenal glands (paired weight) was statistically significantly increased ($p < 0.05$) in Roundup-treated animals when compared to control animals. H&E and Oil Red O staining indicated a moderately higher number of lipid droplets present in the adrenal gland of 10 mg/kg bw/day Roundup-treated rats (Fig. 4).

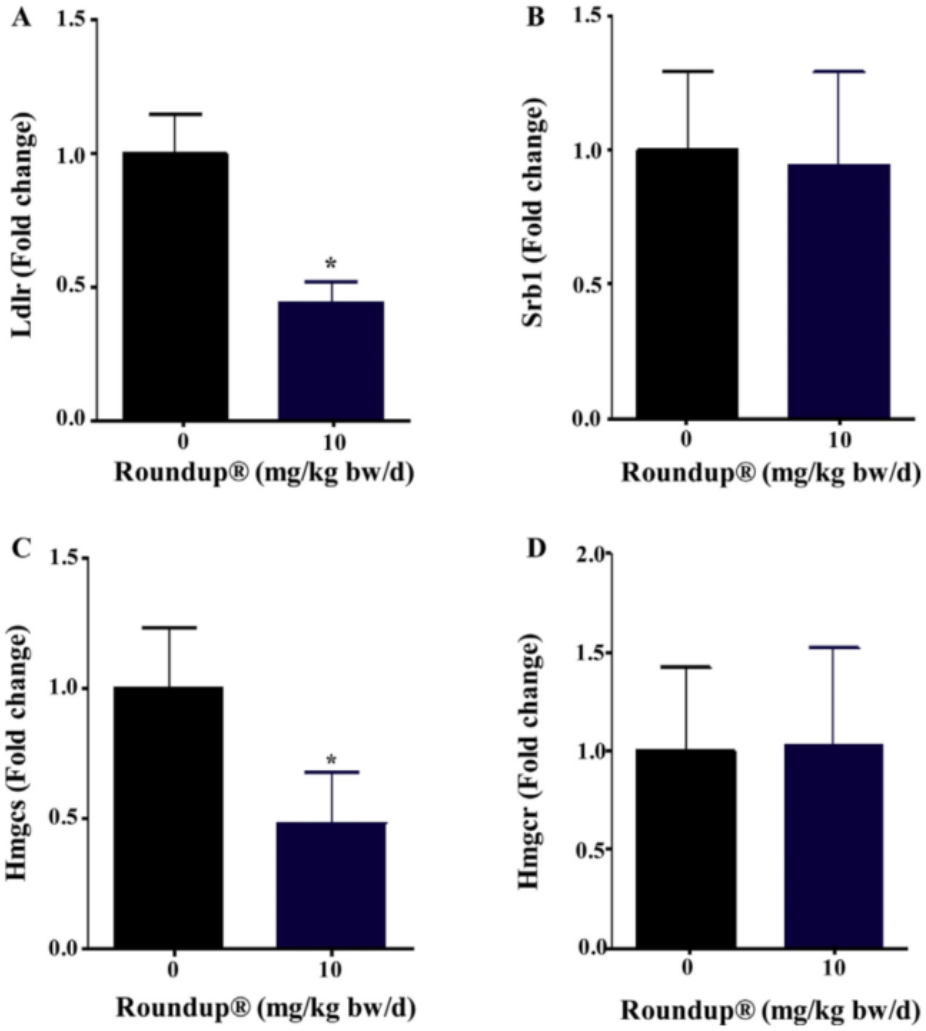
Figure 4. Total cholesterol levels in adrenal gland lysate (A) of vehicle- and Roundup-treated group. The adrenal glands were weighed (B), sectioned and stained for H&E (C) and Oil Red O (D) post Roundup treatment. Sections are representative of two or more runs. Values are presented as mean ± SEM (n = 3-5 per group). 't' test was performed to compare each treatment group with the vehicle group. *, $p < 0.05$.



Effect of Roundup treatment on genes involved in cholesterol intake and de novo synthesis
There was a statistically significant decrease in RNA expression of low-density lipoprotein receptor (Ldlr), a receptor for cholesterol uptake, however, expression of high-density lipoproteins receptor (Srb1) was unaltered in the adrenal gland in Roundup-treated animals when compared to the control group.

Gene expression for enzymes involved in cholesterol *de novo* synthesis was found to be unchanged for Hmgcr but statistically significantly decreased ($p < 0.05$) for Hmgcs in the adrenal gland. Although expression of genes associated with cholesterol mobilization in the gland was decreased, there was a moderately higher accumulation of cholesterol in adrenal cells, which could be due to decreased utilization of cholesterol in Roundup-treated animals (Fig. 5).

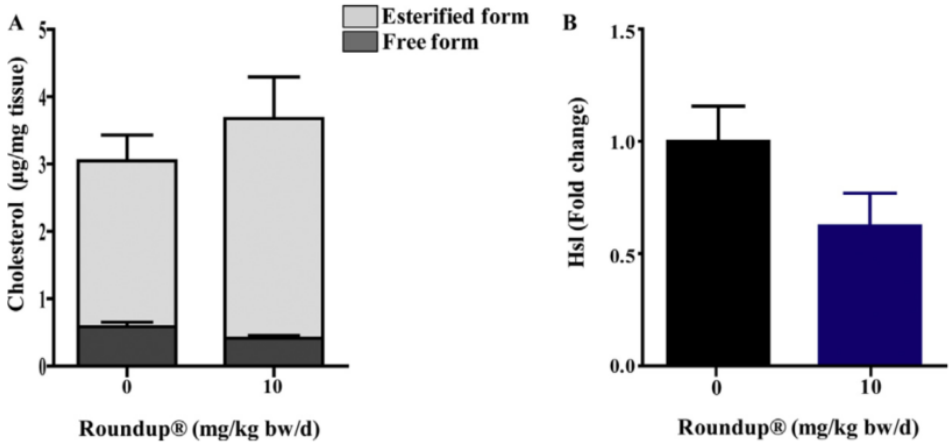
Figure 5. Expression of genes associated with cholesterol import and de novo synthesis in the adrenal gland. qPCR analysis was performed to quantitate the expression of genes involved in cholesterol transport (Srb1, Ldlr) (A and B) and Cholesterol de novo synthesis (Hmgcr, Hmgcs) (C and D). Rpl19 was used as internal control. mRNA expression in the vehicle-treated group was set as 1 fold and the expression in treatment group was calculated in relation to the vehicle group. Data presented as mean ± SEM (n = 5 rats per group). 't' test was performed to compare treatment group from vehicle group. *, significantly different from the vehicle group ($p < 0.05$).



Effect of Roundup treatment on esterified cholesterol (CE) and ester hydrolase
Stored form of cholesterol, i.e. esterified cholesterol, was estimated by calculating free from total cholesterol. There was a tendency towards a moderate increase (statistically not significant) in CE in the adrenal gland of Roundup-treated animals when compared to control animals. Cholesterol ester hydrolase or hormone-sensitive

lipase (Hsl), catalyses the hydrolysis of CE into their free form, was found to be decreased even though changes in both, CE and Hsl, were not statistically significant ($p > 0.05$) (Fig. 6).

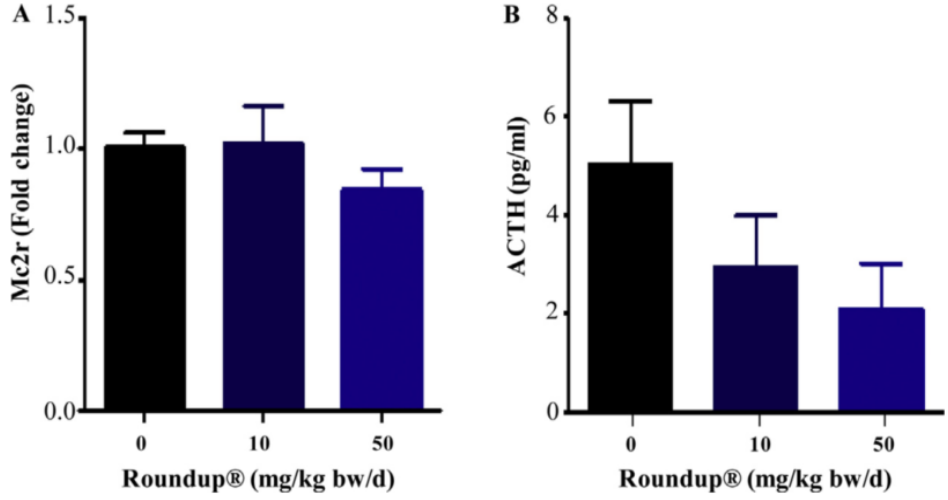
Figure 6. Esterified cholesterol levels of the adrenal gland (A) and gene expression of Hsl in the adrenal gland (B) after treatment with Roundup when compared to control animals. Values are presented in mean ± SEM (n = 6 rats per group). T test was performed to compare the two groups.



Effect of Roundup on adrenal gland ACTH receptor expression and circulatory ACTH levels

There was no change in gene expression of Mc2r, the ACTH receptor in the adrenal gland. However, there was a statistically significant decrease in circulatory levels of ACTH in Roundup-treated rats. The results suggest that Roundup-treatment might have decreased the synthesis or release of ACTH from the pituitary gland. Lower ACTH levels might explain observed downregulation in StAR and Hsl expression, expression of both genes regulated by ACTH in the adrenal gland post Roundup treatment (Fig. 7).

Figure 7. Dose-dependent adrenal ACTH receptor expression (A) and circulatory ACTH levels (B) after treatment of rats with Roundup

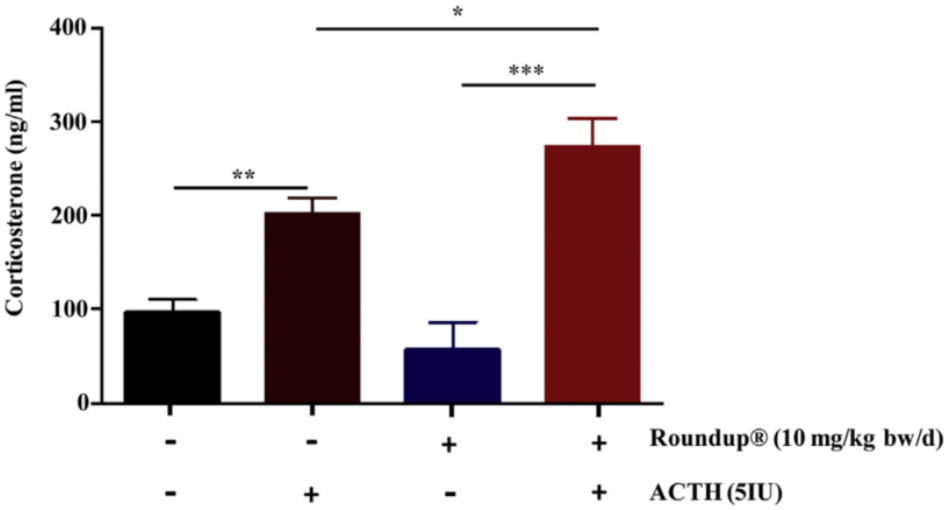


Effect of exogenous ACTH upon circulatory corticosterone levels in Roundup-treated animals

Administration of exogenous ACTH caused a statistically significant increase in corticosterone levels in Roundup-treated rats compared to vehicle-treated rats (Fig. 8). Exogenous ACTH treatment also increased StAR, Hsl expression and the expression of cholesterol homeostasis genes (Srb1, Ldlr, Hmgcr, Hmgcs, Hsl) in the adrenal gland of Roundup-treated rats. The results indicate that responsiveness of the adrenal gland to ACTH was intact in the Roundup-treated rats.

Figure 8. Circulatory corticosterone levels in ACTH administered and/or vehicle and Roundup-treated groups. Animals were treated with vehicle and Roundup (10 mg/kg bw/day). 5 IU of porcine ACTH was administered

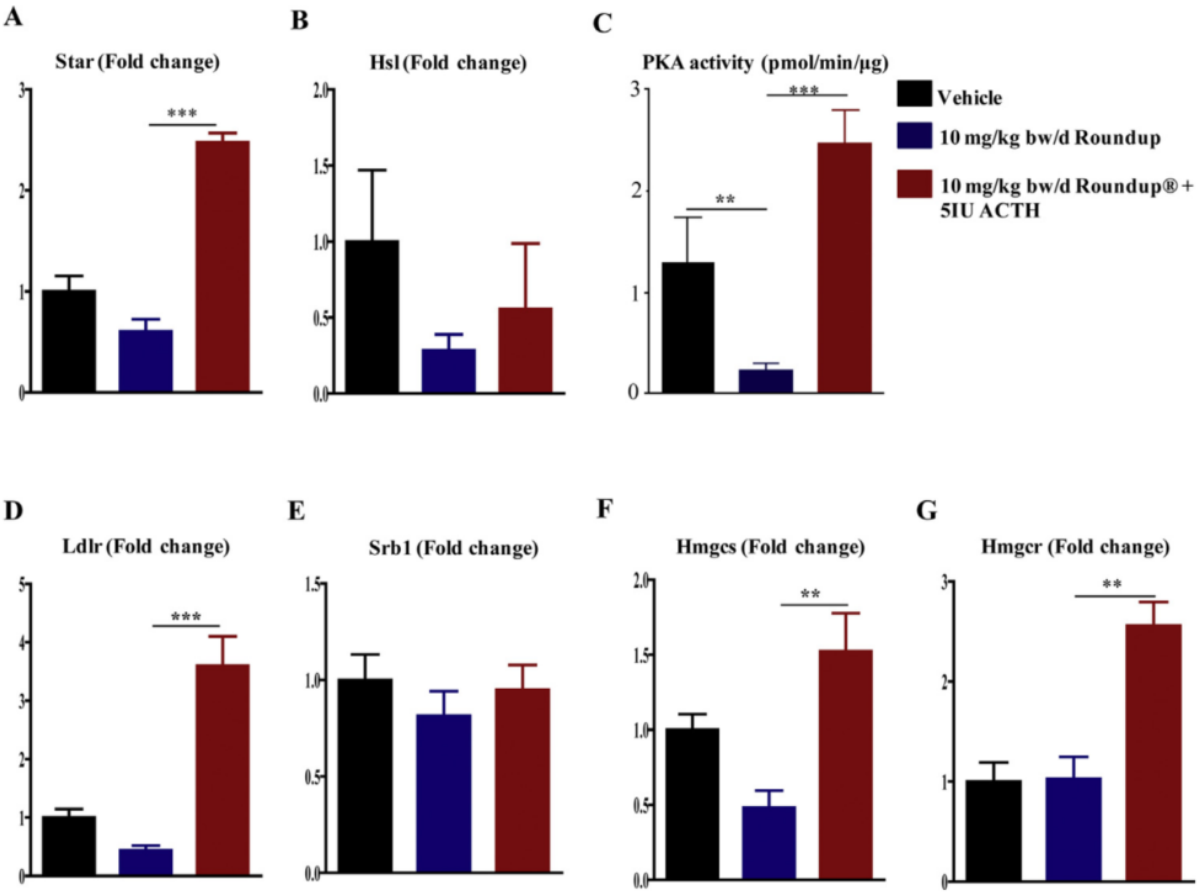
intravenously. Post 1 h of ACTH treatment, corticosterone levels were measured in the plasma. Data presented as mean ± SEM (n = 3-4 rats per group). 't' test was performed to compare vehicle and treatment groups. *, ** and ***, significantly different from the vehicle group by p <0.05. p <0.01, p <0.001 viz.



Effect of Roundup on adrenal gland PKA activity

The cAMP/PKA pathway activated by ACTH in the adrenal gland was examined by quantifying PKA activity. There was a statistically significant decrease in PKA activity in Roundup-treated rats, while exogenous ACTH treatment statistically significantly increased PKA activity (Fig. 9). The results suggested that Roundup decreased endogenous ACTH levels, and in turn cAMP/PKA pathway in the adrenal gland tissue.

Figure 9. Expression of StAR and genes associated with cholesterol homeostasis after treatment with vehicle, Roundup and/or ACTH (Pandey and Rudraiah, 2015). qPCR analysis was performed for StAR (A), Hsl (B), cholesterol homeostasis related genes, Srb1, Ldlr, Hmgcr, Hmgcs (D-G). The cortical region lysate was utilized to quantitate PKA activity (C). Values are presented as mean ± SEM (n = 3-4 rats per group). T test was performed to calculate significance between treatment and vehicle groups. *, ** & ***, significantly different from the vehicle group by p <0.05, p <0.01, p <0.001 viz



Effect of Roundup on cell toxicity

In cancer cell line studies, Roundup has been reported to have an apoptotic effect, therefore the authors investigated one of the makers of apoptosis and performed TUNEL assay in adrenal glands. Immunoblot analysis of cleaved caspase 3 (as a marker of apoptosis) was performed on the adrenal gland tissue lysate of vehicle- and Roundup-treated animals (data not shown). It was observed that cleaved caspase-3 level was up-regulated in rats administered 250 mg/kg bw/day while the levels were unchanged in the 10 mg/kg bw/day treated group. TUNEL assay for in vivo apoptosis was performed to determine DNA fragmentation. The incidence of apoptotic cells in the adrenal gland cortical region was increased in rats of the high dose group. Therefore, decreased corticosterone level observed at the lowest dose level of 10 mg/kg bw/day was not attributed to toxicity or cell death induced by Roundup treatment.

Conclusion

The present study was conducted to examine the EDC effect of Roundup on adrenal gland steroidogenesis and to determine its mechanism of action. For this purpose, after determining the effect of ranges of doses on different parameters such as food consumption, body weight etc., the lowest dose was selected which was devoid of obvious toxic effects other than the endocrine-disrupting effect.

A dose of 50 mg/kg bw/day Roundup manifested significant loss in body weight but not in food consumption, while doses higher than 50 mg/kg bw/day caused a decrease in food consumption as well as body weight of rats in the second week of the treatment. In order to circumvent possible effects of Roundup on causing stress and other toxicity related effects, the doses of Roundup higher than 50 mg/kg bw/day were not used for studying the endocrine-disrupting effect.

There was a statistically significant decrease in circulatory corticosterone levels in the groups treated with 10 and 50 mg/kg bw/day Roundup when compared to the vehicle-treated group. To verify that the decrease in corticosterone is due to possible endocrine effect of Roundup, another steroid hormone, testosterone level was also determined. The result observed suggest suitability of the dose 10 mg/kg bw/day to assess the endocrine-disrupting effect of Roundup.

Roundup-treated animals did not show altered total cholesterol levels in circulation at the lowest dose, i.e. 10 mg/kg bw/day, but it was observed to be moderately higher in the adrenal gland. With this observation, it can be hypothesized that the cholesterol homeostasis within gland may be altered by increased cholesterol intake and/or de novo synthesis. Interestingly, there was downregulation of genes associated with cholesterol intake (Ldlr, Srb1) and de novo synthesis (Hmgcs, Hmgcr). Also, analysis revealed higher levels of esterified or stored form of cholesterol in the adrenal gland of Roundup-treated rats. The data taken together suggest that increased levels of stored cholesterol might be due to lowered utilization and/or lowered hydrolysis of esterified form. Hsl expression was not significantly altered in the present study. Hsl or lipe gene is involved in cholesterol ester hydrolysis and reported to be regulated by cAMP/PKA pathway. Therefore, Roundup appears to act via cAMP/PKA pathway and regulate StAR phosphorylation negatively leading to decrease in cholesterol utilization and increase in cholesterol stored in adrenal glands. Interestingly, increase in the weight of adrenal gland was observed in Roundup-treated animals, but its significance is not clear.

Since Roundup treatment at a dose of 10 mg/kg bw/d decreased corticosterone levels, it became of interest to examine the responsiveness of adrenal gland to exogenous ACTH treatment. The findings that the adrenal gland was responsive to ACTH treatment in Roundup treated animals suggest that Roundup acts at a site higher than the adrenal gland and this indirectly suggests that ACTH synthesis and/or release may be affected. Since the adrenal gland responsiveness to external ACTH was found to be similar or higher compared to vehicle-treated animals suggest that the process of steroidogenesis in the adrenal gland appears to be intact post herbicide treatment. Therefore, it can be inferred that the stimulation of adrenal gland i.e., ACTH synthesis and release appears to be impaired rather than defects in the steroidogenesis machinery of the adrenal gland.

A significantly higher increase in corticosterone levels in response to a supraphysiological dose of ACTH was observed in Roundup-treated rats compared to vehicle-treated rats is perhaps due to higher stored cholesterol content in the adrenal gland of Roundup-treated animals. In sum, the data suggest Roundup appears to act at the hypothalamo-pituitary level under in vivo conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: Formulation tested in vivo (Roundup, 41%, India), which contains polyethoxylated tallow amine (POEA). A non-EU representative formulation was tested.

The data on the formulation are not relevant for the glyphosate EU renewal as the formulation tested in this study contains polyoxyethylene tallow amine (POEA), which is not permitted for use in formulated herbicidal products in the EU.

Further points for clarification:
The relevance of these findings is questionable, and results are not consistent with existing high-quality data, including the OECD multi-laboratory validation of the H295R steroidogenesis assay which included glyphosate alone, not a formulation containing POEA (Hecker M. *et al*., 2011).

Glyphosate was tested as it is a formulation Roundup, 41%, containing POEA, but a non-EU representative formulation was tested. Limited information on the test material was provided, as the CAS No., the purity, batch and source were not indicated. In addition, no historical control data on the analysed parameters under control conditions were provided. The study is considered relevant but to provide supplementary information only.

Technical Comments:
1. The Pandey study in *Toxicology Reports* was poorly designed, lacked proper controls and arrived at an unfounded conclusion. Furthermore, the study failed to demonstrate the alleged impact on hormone synthesis. The doses used in this study are far above any exposures reported for pesticide users or the general population.

2. This study involved administration of a glyphosate-surfactant formulation but the cited LD50 is that for glyphosate alone. The predominant toxicity of oral glyphosate is due to the surfactant.

3. Toxicology studies should include parameters such as animal data, test article preparation and administration, observations (viability and clinical), weekly body weight and food consumption, clinical pathology and histopathology. This study was not conducted according to any structured international guidelines and lacked

this information.

4. The effects observed in this study could be the result of stress and/or the anaesthetic and have nothing to do with exposure to the Roundup branded formulation.

a. Stress is often a by-product of toxicology studies. Some of the stressors in this study include husbandry, handling and dosing of the animals. Therefore, stress-induced effects on data interpretation must be partially mitigated by the study design. This is particularly true in this study as outcomes of the stress response may include decreased total body weights or body weight gain, food consumption and activity; and altered organ weights for selected organs (e.g., decreased thymus and spleen weights or increased adrenal gland weights). Importantly, the authors state "[i]nterestingly, increase in the weight of adrenal gland was observed in Roundup®-treated animals, but its significance is not clear."

b. There are issues with the protocol employed for animal sacrifice. The preferred method of terminating animals for studies evaluating endocrine function is to move them to a holding room separate from the room where the animals will be terminated, transferring only one animal at a time. This helps reduce the animals' stress. There is no indication that this method was employed. Furthermore, decapitation without any form of anaesthesia should be used. Rather, the authors indicate that on day 15 of treatment, the animals were weighed and anaesthetized with 50 mg/kg bw/day pentobarbitone sodium and blood was collected by cardiac puncture. The animals were then sacrificed by cervical dislocation and the adrenal glands were removed, weighed and processed. It is well established that sodium pentobarbitone can affect adrenal gland function. Sodium pentobarbitone inhibited corticosteroid production in rat adrenocortical preparations (Whitehouse B. J. *et al*., 1993).

5. For repeat dosing studies the limit is 1000 mg/kg/day. Therefore, using the LD50 from an acute oral toxicity study as a reference is not relevant. Furthermore, standard practice for dose setting for evaluation of endocrine function is the highest dose level should be at or just below the Maximum Tolerated Dose (MTD) level but need not exceed the limit dose of 1 g/kg/day. A dose level will generally be considered to be at or just below the MTD level if it causes a significant reduction in terminal body weight gain in treated animals vs. controls (no greater than approximately 10%) and no clinical signs of toxicity associated with the dose level are observed throughout the study. The authors stated that "food consumption and body weight were significantly lower beginning at 50 mg/kg/day" and excluded the 100 and 250 mg/kg/day groups from (most of) the analyses due to toxic effects (i.e., body weight and food consumption). However, based on the above guidelines, the 50 mg/kg/day group should have been excluded because there was a marked 19 to 25% decrease in body weights which is a clear indication of overt toxicity.

This uncertainty in distinguishing whether a response is endocrine-related was discussed at the FIFRA SAP meeting that evaluated scientific issues associated with the Weight of Evidence evaluation of the EDSP Tier 1 screening process. In October 2013, the SAP stated that "*In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis."*

6. Additional Assay Deficiencies

a. The number of animals used in the study was not consistent across the groups. The authors claimed to use an n = 5 for 50 mg/kg and n = 10 for the control, 10, 100 and 250 mg/kg/day. However, a varied number of animals were used for each assay/analysis. Depending on the assay, they used 3, 4, 5 or 6 animals. No reason was given for the varying number of animals or which animals were selected for an assay(s) and raises doubts about the meaning of the analysis.

b. Animals in the high dose group were administered a different volume of the Roundup branded formulation compared to the other groups. In general, the test substances should be administered in a constant volume over the range of doses.

c. There was no indication if the animals were fasted overnight prior to the blood and plasma measurements in an effort to reduce variability resulting from non-fasting.

d. Exposure is difficult to estimate due to lack of information on delivery. The Roundup branded formulation was diluted in water (except high dose group); however, it is unclear whether the route of administration was via gavage or in the drinking water.

References:
Hecker M. *et al*. The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter-laboratory validation study. Environ Sci Pollut Res Int (2011), Vol. 18, No. 3, pp. 503-15.

Whitehouse B. J. *et al*. Inhibition of corticosteroid production by sodium pentobarbitone in rat adrenocortical preparations. J Endocrinol (1993), Vol. 136, No. 1, pp. 75-83.

Assessment and conclusion by RMS:

Remarks regarding the study summary as provided by the applicant:

- The following dose levels are indicated: 4.4, 121.9, 244 or 609.8 µL Roundup. However, the low dose level is actually 24.4 µL Roundup. The corresponding dose levels in mg/kg bw/day are correctly indicated in the summary as provided.
- The effect of exogenous ACTH on adrenal gland steroidogenesis was investigated in three additional groups; the second group received ACTG and 10 mg/kg bw/day Roundup.
- In the materials and methods section as provided by the applicant, the part on H&E staining actually contains the text on qPCR analysis. The H&E staining was conducted as follows:

The neutral buffered formaldehyde (NBF) fixed adrenal gland was sectioned (5 µm thick) and stained as reported elsewhere. The sections were mounted in DPX (Sigma-Aldrich Co., Bangalore India) and visualized under light microscope (Olympus IX81 inverted microscope, Tokyo, Japan).

In this study an unknown Roundup formulation was administered to male rats, once daily for 14 days. Varying group sizes were employed, without further justification for this. The final doses selected for further analysis were 10 and 50 mg/kg bw/day, however, the dose of 50 mg/kg bw/day is already considered too high for the purpose of this study as at this dose there was a significant decrease in body weight (-19 to -25% decrease compared to control). The study showed a decrease in circulatory corticosterone levels; no change in total cholesterol levels was seen in the circulation but was moderately higher in the adrenal gland after treatment with Roundup. There was a downregulation of genes associated with cholesterol intake and de novo synthesis. In addition, higher level of esterified or stored form of cholesterol in the adrenal gland was seen in treated rats. The responsiveness of the adrenal gland to external ACTG was similar or higher compared to vehicle treated animals suggesting the process of steroidogenesis in the adrenal gland appears to be intact post Roundup treatment.

The study is considered as supplementary data only. The study is carried out with a formulation of glyphosate, thus side effects caused by co-formulants cannot be excluded. According to the applicant, the tested formulation contains polyethoxylated tallow amine (POEA), which means a non-EU representative formulation was tested.
The study is considered reliable with restrictions because of the following reasons: the study was not conducted according to any international guideline, no GLP status, the test substance is not sufficiently characterised (particularly, purity and batch not specified), low and varying number of animals, acclimatisation period not reported, different dosing volume used between groups, clinical observations not presented, individual data missing, no historical control data and positive control missing.

B.6.8.3.21. Public literature – Growth of ERα positive cholangiocarcinoma cells via non-genomic ER/ERK1/2 signaling pathway (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.8.3
Report author	Sritana N. *et al*.
Report year	2018
Report title	Glyphosate induces growth of estrogen receptor alpha positive cholangiocarcinoma cells via non-genomic estrogen receptor/ERK1/2 signaling pathway
Document source	Food and Chemical Toxicology (2018), Vol. 118, pp. 595-607

Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

Previous studies showed that glyphosate is able to stimulate cholangiocarcinoma (CCA) cell growth of HuCCA-1 cells via the oestrogen receptors ERα in the same concentration range as estradiol (E2) is able to stimulate their cell growth via the receptor ERα. The present study investigated the effect of glyphosate on the oestrogen-signalling pathway involved in the induction of CCA cell growth and the effect of glyphosate on the expression of proliferative signalling-related proteins including ERα, VEGFR2, pERK, PI3K(p85), and PCNA. Additionally, the effects of glyphosate on cell growth, cell cycle and molecular signalling pathways were measured.

Materials and methods

Test Material:	Glyphosate (>98%)
Origin:	AccuStandard (New Haven, USA)
Lot/Batch number:	Not reported
Purity:	Not reported
CAS#:	Not reported

Chemicals

Substance	Function	Sponsor
17β-estradiol (E2)		Sigma-Aldrich (Sigma-Aldrich, USA)
(Z)-4-hydroxytamoxifen	ER antagonist	Tocris Bioscience (Tocris Bioscience, UK)
U0126	MEK1/2 inhibitor	Cell signaling Technology Inc. (USA)

Cell lines

Cell line	Origin	Sponsor
HuCCA-1	bile duct tumour mass from Thai	Professor Stitaya Sirisinha
RMCCA-1	CCA patients	Dr. Kawin Leelawat
MMNK-1	normal cholangiocytes	Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan)
MCF-7	breast cancer cell line	American Type Culture Collection (ATCC, USA)

Cell culture conditions

Cells	Medium	Condition
HuCCA-1 RMCCA-1	HAM's F-12 medium supplemented with 10% foetal bovine serum (FBS) (JR Scientific Inc., USA), 2 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin (Gibco, USA)	37 °C in a 5% CO_2 humidified atmosphere
MMNK-1	DMEM medium supplemented with 10% FBS, 2 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin (Gibco, USA)	
MCF-7	MEM media supplemented with 10% FBS, 2 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin, 1% v/v non-essential amino acids, 1 mM sodium pyruvate and 10 mg/L insulin	

After the confluence of the cells (approximately 80%), cells were sub-cultured. The culture plates were then maintained in a 5% CO_2 humidified incubator at 37°C.

Cell culture and treatment conditions
For the oestrogen withdrawal condition, cells were cultured in 100 mm sterile plates and allowed to reach approximately 60% confluence. Cells were washed twice with sterile PBS. Next, cells were cultured in 10% dextran-charcoal stripped FBS (CSS) (HyClone, USA) in a non-phenol red RPMI medium (Sigma, USA) containing all supplementations except FBS for 4 days prior to seeding. After 24 h incubation for cell attachment, the cells were treated with various concentrations of test compounds and positive controls; 17β-estradiol (E2), in non-phenol red RPMI medium containing 5% CSS with all supplements according to our previous study. For serum-free treatment conditions, after seeding and incubation for 24 h for cell attachment, cells were cultured in serum-free medium (SF) 24 h prior to treatment.

Cell viability
PrestoBlue (Invitrogen Corp., USA) reagent is quickly reduced by metabolically active cells, providing a quantitative measure of viability and cytotoxicity. Before cell seeding for the PrestoBlue assay, cells were cultured in oestrogen-withdrawal conditions. After treatment, 10 µL of PrestoBlue reagent was added into each well and incubated at 37°C for 30 min. The fluorescence was measured at an excitation/emission wavelength of 560/590 nm using a SpectroMax M3 microplate reader (Molecular Devices, USA), and expressed as the percentage of cell viability compared to controls. The remaining media was then removed and 100 µL of 500 µg/mL of MTT-containing medium was added to each well. Cells were incubated for 4 h and then lysed with dimethyl sulfoxide (Sigma-Aldrich, USA). The optical density (OD) was measured at 570 nm with a reference wavelength of 650 nm using a SpectroMax M3 microplate reader, and was expressed as the percentage of cell viability compared to controls.

Cell cycle analysis
After maintaining in oestrogen-withdrawal conditions, CCA cells were seeded into 60 mm plates (1.5×10^6 cells/plate) and cultured overnight. The cells were then treated with test compounds and cultured for another 48 h. The cells were trypsinized with phenol red-free trypsin (Gibco, USA) and washed with cold phosphate buffer saline (PBS). Subsequently, cells were fixed with cold 70% ethanol and incubated at 4°C for 1 h and then washed with PBS. Cells were stained by adding 1 mL of 50 g/mL propidium iodide (PI) solution (Sigma-Aldrich, USA) and 0.5 ng/mL RNAse (Sigma-Aldrich, USA). Analysis was performed with a BD FACSCanto flow cytometer (BD Biosciences, USA) and cell cycle distributions were analysed by the ModFit LT software (Verity House Software, USA).

mRNA expression analysis
Quantitative real-time RT-PCR (qRT-PCR) was used to determine the mRNA expression of several genes, including ERα, ERβ1, ERβ2, ps2, progesterone receptor (PR) and β-actin. Total RNA was extracted using the RNAeasy mini kit (QIAGEN, Germany) according to instructions by the manufacturer. The remaining residual DNA in RNA samples was removed with RNase-Free DNase (QIAGEN, Germany). Quantitative real-time PCR was performed using RNA-direct SYBR Green Realtime PCR Master Mix (Toyobo, Japan) in an Applied Biosystems StepOnesPlus Real-time PCR (Life Technologies, USA).

Western blot analysis
Cell lysates were prepared by placing cells in lysis buffer. Then cell lysates were sonicated for 30 min at 4°C before centrifugation at 14,000 rpm for 15 min at 4°C. The protein concentrations were determined by the Bradford reagent (Bio-rad, USA). Equal amounts of total protein (50 µg) from each sample were loaded on 7.5% SDS-polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes (Pall Corporation, USA). The membranes were blocked in 5% non-fat milk in tris-buffered saline (TBS) containing 0.1% tween-20 for 1 h at room temperature and probed with primary antibodies diluted in TBST containing 5% non-fat milk at 4°C overnight. The dilution of primary antibodies are as follows:

ERα	1:1000
ERβ	1:6000 (Merck, USA)
pERK	1:1000
ERK	1:4000
pP38	1:1000
P38	1:2000
cyclin D1	1:500
cyclin B1	1:1000
cyclin E1	1:1000 (Cell signaling, USA)
cyclin A	1:500 (BD Biosciences, USA)

pERa-Ser118	1:1000
VEGFR2	1:1000 (Santa Cruz Biotechnology, USA)
Beta-actin	1:20,000 (Bio-Rad, USA)

Membranes were then washed and incubated with the secondary antibody coupled with HR-peroxidase (1:2000 dilution in blocking buffer containing 0.1% Tween-20) for 2 h.

Immunoreactivity was visualized using enhanced chemiluminescence detection (ECL Kit) according to the manufacturer's recommendations (GE Healthcare, UK) and exposed to ECL X-ray film (Amersham, Biosciences, UK).

Immunofluorescent staining
For immunofluorescent staining, cells were grown in complete medium conditions and cultured until reach to 80%. Then, HuCCA-1 and MCF-7 cells were seeded on coverslips in 24-well plates (1.5×10^4 cells/well) and incubated overnight for cell attachment. Cells were then washed twice with PBS, fixed in 4% paraformaldehyde/PBS for 10 min at 4 °C, subsequently washed and permeabilised with blocking buffer (1× PBS, 1% BSA and 0.2% Triton-X100) for 45 min at room temperature. Cells were subsequently counterstained with anti-rabbit ERα clone 60C (Merck, USA) (dilution 1:500 and 1:1000 for HuCCA-1 and MCF-7, respectively) for 1 h. Next, cells were washed and blocked with blocking buffer for 10 min at room temperature. Cells were incubated at room temperature with the anti-rabbit Rhodamine Red™ IgG secondary antibody (1:400, Jackson ImmunoResearch Laboratories, USA) and counterstained with Hoechst 33342 (nuclei staining; Molecular probes, USA) for 45 min in the dark at room temperature. Cells were then washed twice with PBS. Finally, cells were mounted with mounting media onto a microscopy slide and images were captured by ImageXpress High Content Imaging System and analysed with MetaXpress analysis software (Molecular Devices, USA). The experiments were performed in triplicates with the different cell passages.

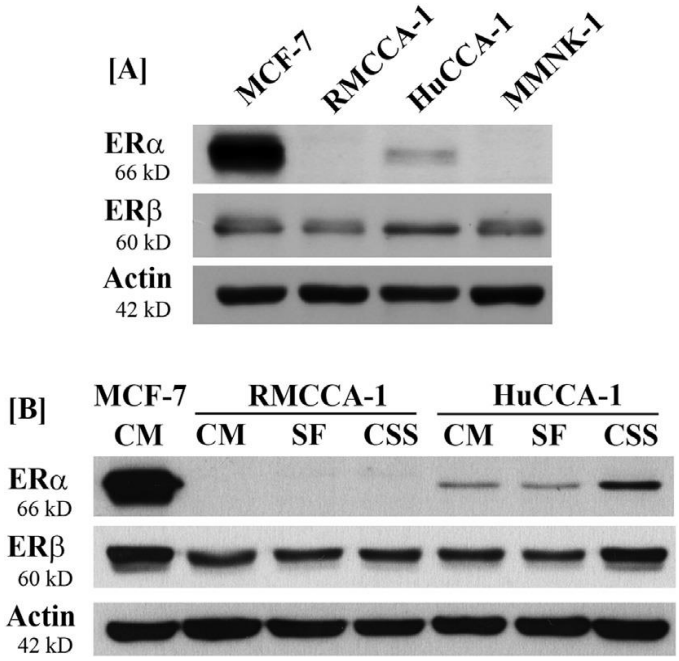
Statistical analysis
All experiments were repeated at least three times with freshly prepared chemicals in each experiment and applied to treat the cell lines in different passages. Data are presented as the means ± standard error (SEM). For cell viability assay, unpaired t-tests were used for comparison between two groups. For protein and mRNA expressions, one-way ANOVA was used for statistic comparison between controls and chemical treatments. For multiple comparisons of data among controls, treatment with and without inhibitors, two-way ANOVA was used to determine statistic comparison. In all cases, a p-value less than 0.05 was considered as statistically significant.

Results

Variation of oestrogen receptors (ERs) in cholangiocytes, CCA and breast cancer cells
The initial experiment was performed to determine the expression levels of ERs in MMNK-1, HuCCA-1 and RMCCA-1 cells and compared to that in MCF-7 human breast cancer cells. The results showed that HuCCA-1 cells expressed ERα but the expression level was much lower than that in MCF-7 cells. Interestingly, ERα was not detected in RMCCA-1 and MMNK-1 cells. Meanwhile, ERβ was expressed in all cell lines at similar levels.

Moreover, the protein level of the ERs was compared among the different culture conditions: CCA cells in complete medium with 10% serum (CM), serum-free medium (SF) and charcoal-stripped serum medium (CSS). HuCCA-1 cells displayed an increased ERα protein expression in hormone withdrawal medium but not in other conditions. These data suggest that the responses to oestrogen at the level of ERs expression of MMNK-1, RMCCA-1 and HuCCA-1 cells might be different because these cells had different patterns of ERs expression.

Figure 1. Protein levels of oestrogen receptors (ERs), including ERα and ERβ, in breast cancer (MCF-7), intrahepatic cholangiocarcinoma (RMCCA-1 and HuCCA-1) and cholangiocyte (MMNK-1) cell lines.
[A] The comparison of protein expression among the four cell lines cultured in complete medium (10% FBS) conditions (basal level of cell culture condition).
[B] Cells were cultured in complete medium with 10% FBS (CM), 2 days of serum-free medium (SF) and 5 days of 10% charcoal-stripped serum medium (CSS). The hormone-dependent breast cancer cell line, MCF-7, was used as a positive control.

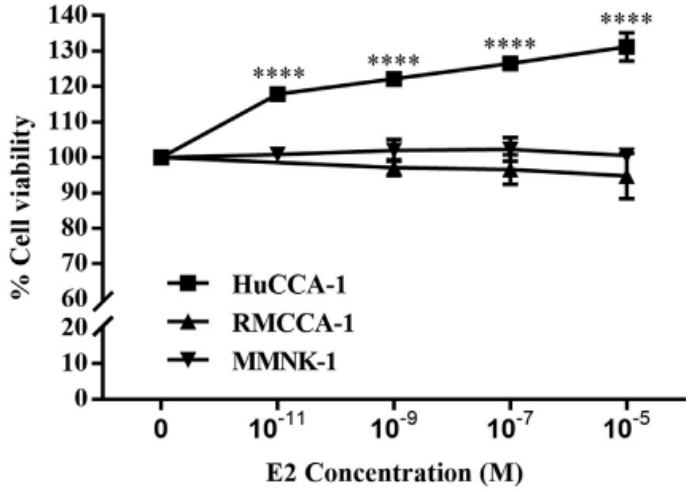


A qRT-PCR was performed to determine the mRNA expression of the ERα, ERβ1, ERβ2, ps2 and PR genes. The results showed that under normal culture conditions (with 10% FBS), all cell types expressed ERs mRNA but in different levels. The results showed that the MCF-7 cells had the highest ERα mRNA expression level, followed by the HuCCA-1 and RMCCA-1 cells. There was no difference in ERβ1 mRNA expression in all cell types while HuCCA-1 cells had the lowest level of ERβ2 mRNA expression when compared among the cancer cells.

Cell proliferation following oestrogen treatment in cholangiocytes and CCA cells
Different levels of ERs protein and mRNA expression were observed among the MMNK-1, RMCCA-1 and HuCCA-1 cell lines. Because of this, it could be possible that the responses of each cell following treatment with oestrogen (E2) might be different. To prove this, the cells were treated with various concentrations of E2 under oestrogen withdrawal conditions.

It was observed, that only the HuCCA-1 cells underwent a significant increase in cell proliferation after E2 treatment, in a dose-dependent manner, while no such change was noticeable in the other cell types. These data suggest that ERα might be the main target of E2-induced cell proliferation since the HuCCA-1 cells were the only one that expressed ERα while none of the other tested cells.

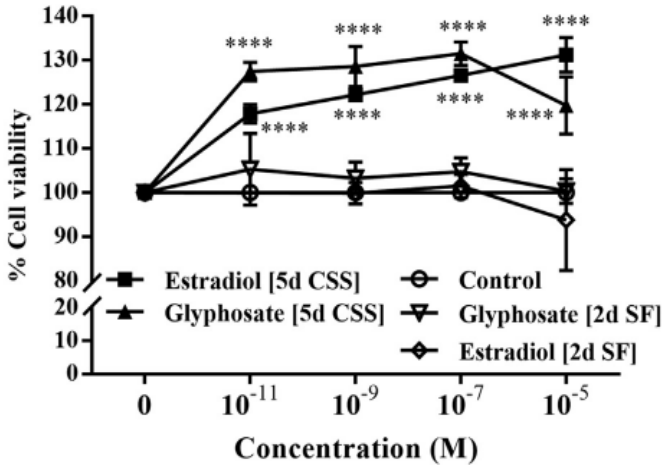
Figure 2. The effects of estradiol (E2) and glyphosate on cell viability.
Effects of E2 on RMCCA-1, HuCCA-1 and MMNK-1 cell viability in hormone withdrawal medium condition. The cells were grown in 10% charcoal-stripped serum medium (CSS) for 5 days and treated with 10^{-11} to 10^{-5} M of E2 for 48 h in 5% CSS medium.



Glyphosate and E2-induced HuCCA-1 cell proliferation
Additionally, it was figured out if glyphosate might induce CCA cell proliferation via oestrogen signalling. To achieve this, a cell viability assay of ERα-expressing CCA cell line, HuCCA-1, was performed under oestrogen-withdrawal conditions.

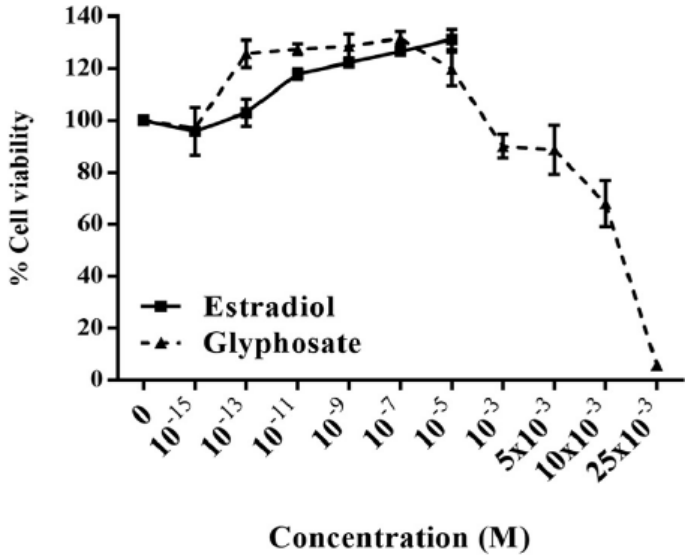
The results showed that both E2 and glyphosate exerted the proliferative effects in these cells only in the CSS condition. Thus, we used CSS conditions in all further subsequent experiments.

Figure 3. The effects of estradiol (E2) and glyphosate on cell viability.
HuCCA-1 cells were cultured in 2 different conditions prior to treatment: serum-free medium for 2 days (2d SF) and 10% charcoal-stripped serum medium for 5 days (5d CSS) followed by treatment with 10^{-11} to 10^{-5} M of estradiol or glyphosate for 48 h.



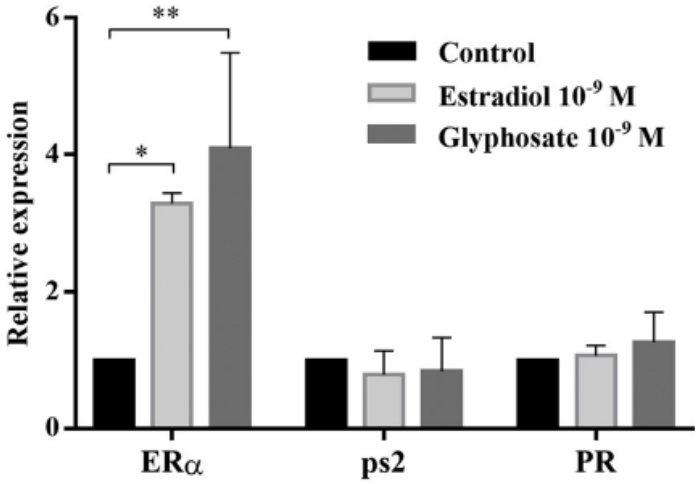
In addition, HuCCA-1 cells were treated with various concentrations of glyphosate and E2. After 48 h of incubation, cell viability analysis was carried out through the Prestoblue assay. The results showed that the low concentrations of glyphosate and E2 significantly induced HuCCA-1 cell proliferation. These data suggested that glyphosate might induce HuCCA-1 cell proliferation via the oestrogen signalling pathway.

Figure 4. The effects of estradiol (E2) and glyphosate on cell viability.
HuCCA-1 cells were treated with 10^{-15} to 10^{-5} M of estradiol or 10^{-15} to 2.5×10^{-2} of glyphosate for 48 h in 5% CSS medium.



Glyphosate and E2 induce ERα mRNA expression but not in ERE responsive genes
The oestrogen signalling pathway was previously categorized into genomic and non-genomic signalling. The results showed that glyphosate and E2 induced only ERα mRNA expression, but did not result in significant changed in ps2 and PR mRNA levels. These data suggested that glyphosate and E2 might not have effects mediated through the genomic/classical of the oestrogen signalling pathway.

Figure 5. The relative mRNA levels of genes that involve with oestrogen signalling pathway.
Relative mRNA levels of ERα, ps2 and PR in HuCCA-1 cells after incubation with E2 and glyphosate at a concentration of 10^{-9} M. HuCCA-1 cells were grown in non-phenol red RPMI containing 10% CSS medium for 5 days prior to treatment. (* = p ≤0.05, ** = p ≤0.01, **** = p <0.0001, significantly different as compared to controls).



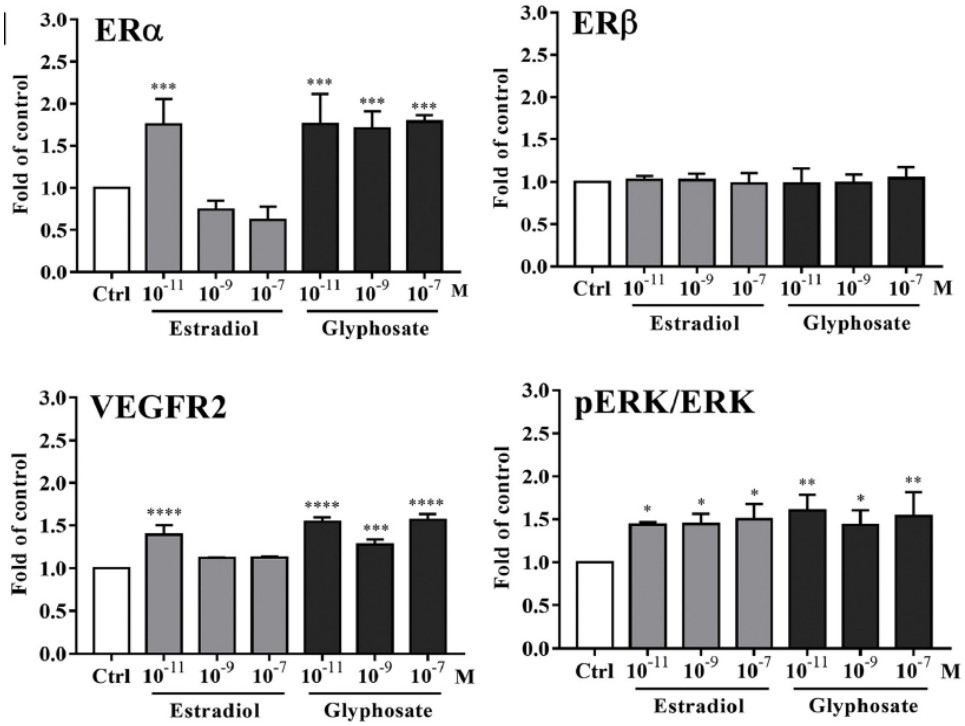
Glyphosate and E2 effects on various signalling protein expressions in CCA cells
In addition, the expressions of signalling proteins related to the non-genomic signalling pathway of oestrogen were analysed.

The results demonstrated that glyphosate and E2 altered the expression levels of several signalling proteins, including ERα, ERβ, VEGFR2, phosphorylated ERK (pERK) and ERK.

At 48 h of exposure, all tested concentrations of glyphosate increased the levels of ERα while only 10^{-11} M E2 induced ERα expression.

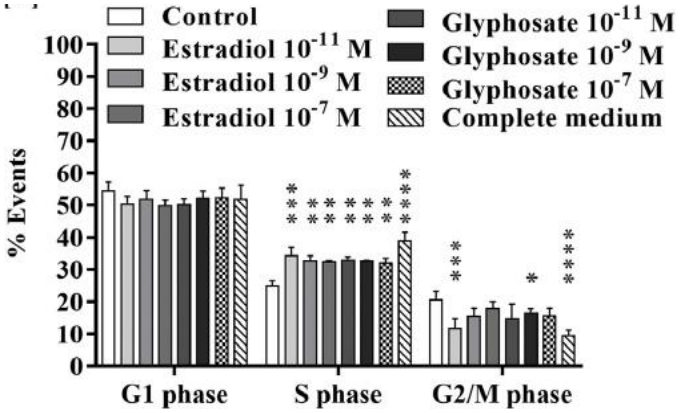
These results suggest that glyphosate altered the expression of ERα and other signalling proteins related to the non-genomic signalling pathway of oestrogen, especially the ERK1/2 pathway.

Figure 6. Effects of estradiol and glyphosate on the level of protein expression in E2-withdrawal HuCCA-1 cell culture.
48 h incubation time resulted in specific bands for ERα, ERβ, VEGFR2, phosphorylated-ERK (pERK), total ERK and β-actin optical densities of specific bands were determined from western blots, and each band was normalized to the β-actin band. The normalized mean of three replications ± SE optical density values are shown in the histogram for ERα, ERβ, VEGFR2, p-ERK/ERK (n = 3, * = p ≤0.05, ** = p ≤ 0.01, *** = p ≤ 0.001, **** = p <0.0001, significantly different as compared to controls).



Cell cycle distribution effects after treatment with E2 and glyphosate
After 48 h of E2-depleted treatment with E2 and glyphosate, the cell cycle was measured by using flow cytometry and PI staining, the results demonstrated that both chemicals in all tested concentrations increased cells in the S-phase.

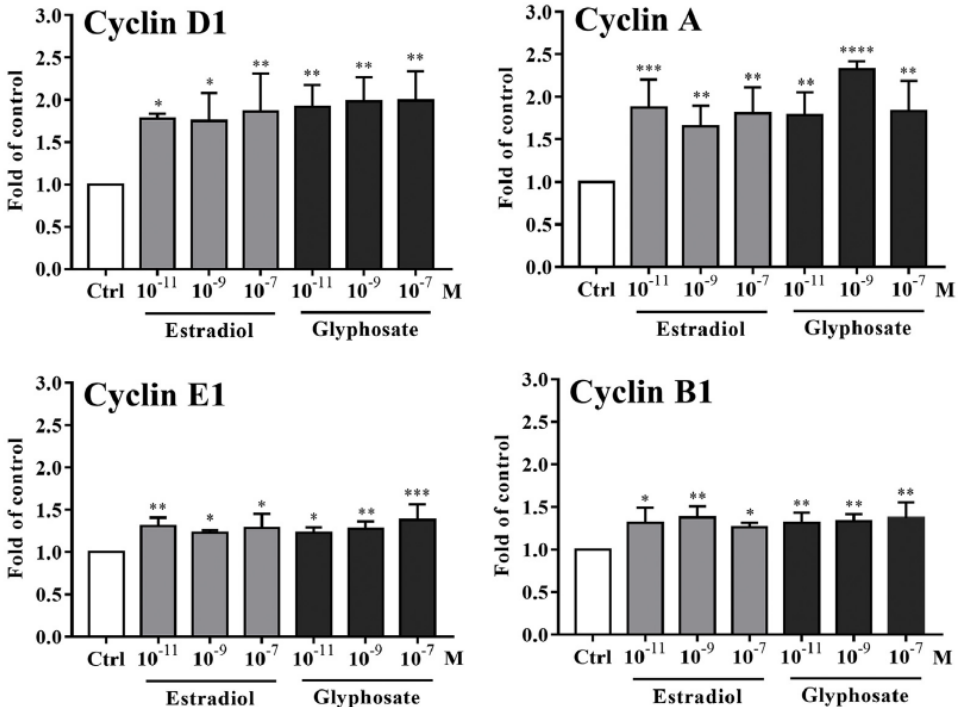
Figure 7. The demonstration of a concentration-effect on cell cycle by estradiol and glyphosate in HuCCA-1 cells. [A] Cells were treated with varying concentrations (10^{-11} to 10^{-5} M) of estradiol and glyphosate. Cell cycle distributions were compared in hormone withdrawal medium (CSS medium) by using PI staining flow cytometry at 48 h; significantly different as compared to controls.



Glyphosate and E2-induced the levels of cyclin signalling protein expressions

Glyphosate and E2 increased S-phase cell cycle distribution. Therefore, the expression of cyclin signalling proteins involved in cell cycle regulation was analysed. The results showed that expressions of all cyclins were increased after 48 h of exposure, especially cyclin D1 and cyclin A, which are involved in driving the cells to the S-phase.

Figure 8. The effects of estradiol and glyphosate on the level of cell cycle-regulated protein expressions in E2-withdrawal HuCCA-1 cell culture. 48 h incubation time resulted in specific bands for cyclin D1, cyclin A, cyclin E1, cyclin B1 and β-actin (representative sample from one experiment). Optical densities of specific bands were determined from the western blot and each band was normalized to the β-actin band. The normalized mean of three replications ± SE optical density values are shown in the histogram for ERα, ERβ, VEGFR2, p-ERK/ERK (n = 3, * = p ≤0.05, ** = p ≤0.01, *** = p ≤0.001, **** = p <0.0001, significantly different as compared to controls).



The proliferative effects of glyphosate are mediated via oestrogen receptors
HuCCA-1 cells were further studied using an ER antagonist, 4-hydroxytamoxifen, which was previously reported to inhibit the growth of human CCA cells.

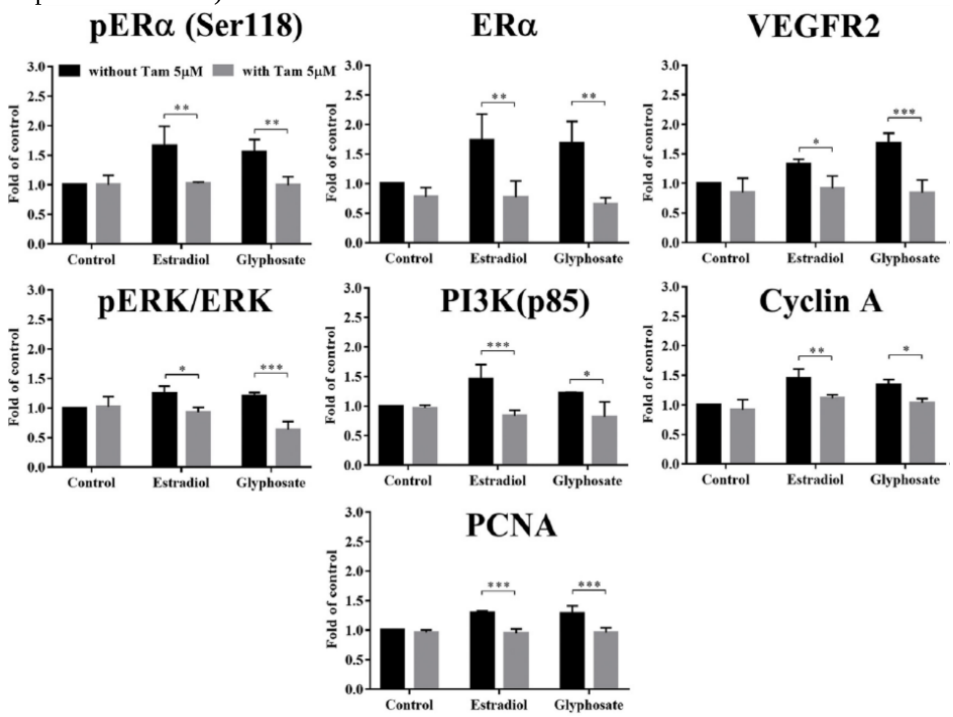
The results showed that 4-hydroxytamoxifen inhibited the proliferative effects of glyphosate and E2. Furthermore, 4-hydroxytamoxifen inhibited the induction of S-phase cell populations by glyphosate and E2.

The expression of several signalling proteins after treatment with glyphosate, E2 and 4-hydroxytamoxifen was also determined.

The results showed that after co-treatment the chemicals with ER antagonist, 4-hydroxytamoxifen, all signalling proteins that involved with cell proliferation and non-genomic signalling of oestrogen, including pERα (Ser118), ERα, VEGFR2, pERK/ERK, PI3K (p85), cyclin A and PCNA, had decreased levels of expression compared to chemicals treatment alone. These data suggest that the effects of glyphosate occurred via oestrogen signalling in the HuCCA-1 cells.

Figure 9. Effects of an ER antagonist (4-hydroxytamoxifen, Tam) on the levels of protein expressions after co-treatment with E2 and glyphosate (10−11 M) in E2- withdrawal HuCCA-1 cell culture. 48 h incubation time resulted in specific bands for ERα, VEGFR2, phosphorylated-ERK (pERK), total ERK, PI3K(p85), cyclin A, PCNA and β-actin (representative sample from one experiment). Optical densities of the specific bands were determined from the western blot and each band was normalized to the β-actin band. The normalized mean of three replications ± SE optical density values are shown in the histogram for ERα, ERβ, VEGFR2 and

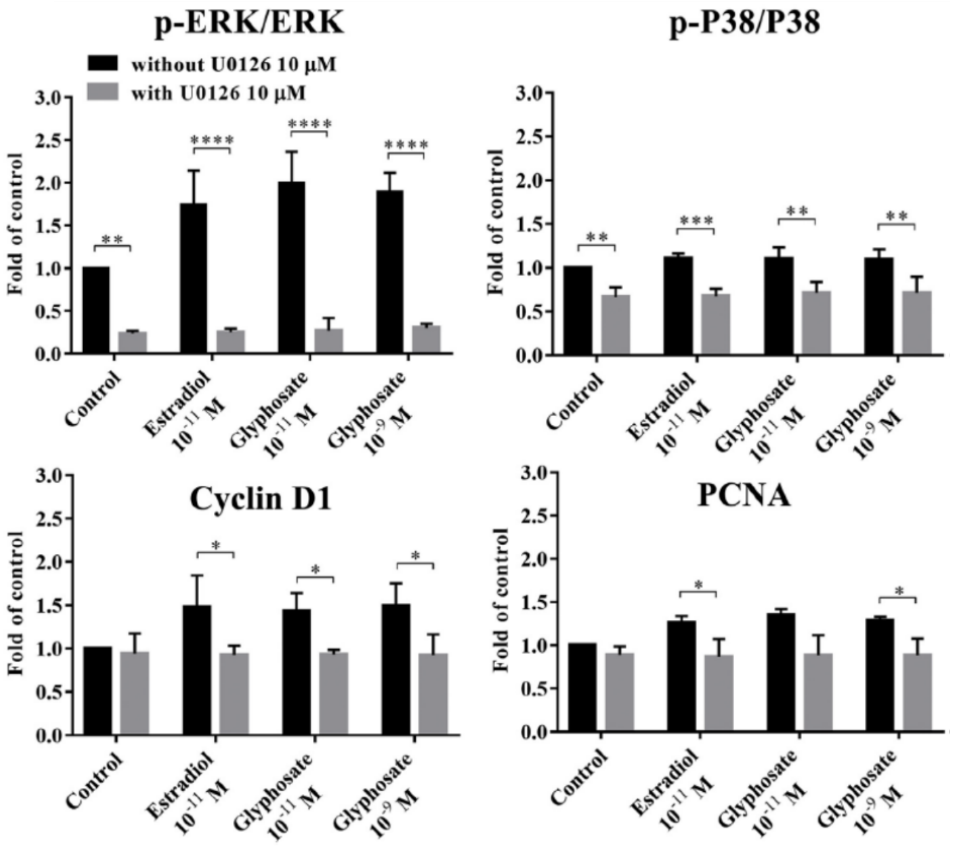
pERK/ERK (n = 3, * = p ≤0.05, ** = p ≤0.01, *** = p ≤0.001, **** = p <0.0001, significantly different as compared to controls).



Glyphosate effects on HuCCA-1 were partly mediated through the MEK signalling pathway
This study already revealed that glyphosate induces CCA cell proliferation and altered expression levels of several signalling proteins, such as pERK, cyclin D1 and cyclin A. Next, a MEK1/2 inhibitor (10 μM U0126) was used to determine whether this signalling pathway is related to glyphosate effects or not.

The results showed that U0126 decreased the expression level of several proteins, including pERK, pP38 and cyclin D1. The reduction of PCNA suggested that U0126 inhibited the proliferation effect of both E2 and glyphosate. These data indicated that the effects of glyphosate were partly mediated through the MEK signalling pathway.

Figure 10. The effects of glyphosate on signalling protein expression. E2-withdrawal HuCCA-1 cells were used. [A] 10 μM of MEK inhibitor (U0126) was applied for co-incubation with HuCCA-1 cells. 48 h incubation resulted in specific bands for p-ERK, ERK, p-P38, P38, cyclin D1, PCNA and β-actin (representative sample from one experiment). Optical densities of specific bands were determined from the western blot and each band was normalized to the β-actin band. [B] The normalized mean of three replications ± SE optical density values are shown in the histogram for expression of different proteins (n = 3, * = p ≤0.05, ** = p ≤0.01, *** = p ≤0.001, **** = p <0.0001, significantly different as compared between with and without inhibitor co-treatment).

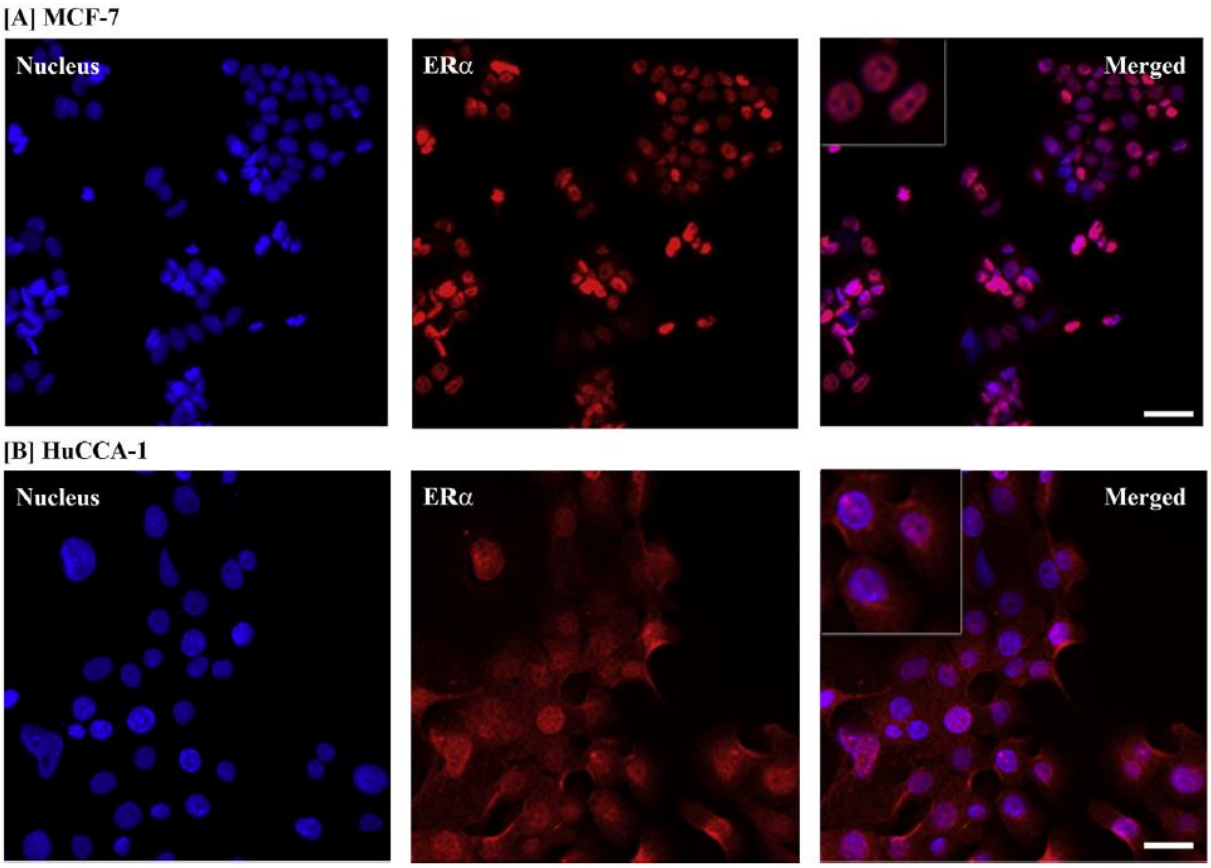


Different localization of ERα between HuCCA-1 and MCF-7

To determine ERα localization in CCA and breast cancer cells, HuCCA-1 cells and MCF-7 were grown in complete medium conditions on a coverslip and examined by immunofluorescent staining technique. The result showed a punctate ERα expression that was mainly localized in the cytoplasmic compartment up to 89.1 ± 7.5% in HuCCA-1 cells, whereas 88.7 ± 2.3% was primarily localized in the cell nucleus in MCF-7 cells. The cytoplasmic marker α-tubulin antibody was also co-stained to ensure the subcellular localization of the ERα in the cytoplasm of the cell.

The results indicated that the oestrogen-signalling pathway of HuCCA-1 cells might be related to non-genomic action, while oestrogen-signalling pathway of MCF-7 cells is a mainly genomic mechanism.

Figure 11. Detection of cytoplasmic and nuclear ERα expression by immunofluorescent staining. ERα expression and localization in MCF-7 [A] and HuCCA-1 [B] cells, (Rhodamine red, red). Cell nuclei were stained by Hoechst 33342 (blue). Images were captured at 40× magnification. Scale bar was at 20 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the online version of this article.)



Conclusion

According to the data in the conducted study, a possible mechanism of glyphosate-induced cell proliferation via oestrogen receptor signalling can be proposed. Glyphosate may bind to ERα followed by an activation step that starts with the phosphorylation of ERα and activation of other signalling proteins by phosphorylation or some other mechanisms. The signalling proteins involved in this mechanism include ERK, PI3K(p85), cyclin D1 and cyclin A. The results of oestrogen signalling activation may induce gene and protein expression of other proteins, include ERα, VEGFR2, and PCNA.

Chemicals that can modulate oestrogen receptor activities could contribute as a risk factor of ethology and progression of the cancers. There are high risks of glyphosate contamination in the environment and increased risk of exposure to this chemical.

The results obtained from the present study explain, at least in part, how glyphosate act on the oestrogen-signalling pathway in inducing proliferation of cholangiocarcinoma cells.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: The glyphosate and other chemicals evaluated in the study were not analytically verified. No batch, purity or CAS No. of the test substances and chemicals reported. No historical control data given. The results showed that glyphosate has a similar potency as estradiol (E2) when tested at extremely low concentrations These specific findings have not been corroborated by other ED studies. This publication is considered unreliable.

Assessment and conclusion by RMS:

In this study the effect of glyphosate on the estrogen signaling pathway involved in the induction of CCA cell growth was investigated. The effect on cell growth, cell cycle and molecular signaling pathways were measured.

Estradiol induced cell proliferation in HuCCA-1, but not in RMCCA-1 and MMNK-1 cells. Glyphosate also induced HuCCA-1 cell proliferation. After treatment with glyphosate or estradiol, the S-phase of the cell cycle and protein levels of the cyclin family were increased. Both compounds also induced the expression of proliferative signaling-related proteins including ERα, VEGFR2, pERK, P13K(p85) and PCNA. The effects of glyphosate and estradiol were abolished by the ER-antagonist 4-hydroxytamoxifen.

The study is considered as reliable with restrictions by the RMS. No specific information on the glyphosate used in this study is given (no batch, CAS no.). Furthermore, no GLP status was indicated, test substances were not sufficiently characterized and no historical control data were given.

B.6.8.3.22. Public literature – Effects on testosterone synthesis in male rats (study for which the RMS requested a summary in order to further justify the categorization)

1. Information on the study

Data point:	CA 5.8.3
Report author	Zhao H. *et al*.
Report year	2018
Report title	Effects of glyphosate on testosterone synthesis in male rats
Document source	Asian Journal of Ecotoxicology (2018), Vol. 13, No. 5, pp. 242-247
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

The paper aims to investigate the effect of subchronic glyphosate exposure on testosterone synthesis in male rats. Male SD rats were randomly divided into control group (0 mg/kg), low dose group (50 mg/kg), middle dose group (200 mg/kg) and high dose group (800 mg/kg), which were administered to glyphosate by gavage. At 4th, 8th and 12th week, a subunit of animals in each group was killed, and blood and organs were collected. The serum content of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T) and estradiol (E2) were measured by radioimmunoassay. The histopathological changes of testis were observed with haematoxylin-eosin staining. The expression of 3-beta-hydroxysteroid dehydrogenase (3β-HSD), steroid synthesis of acute regulatory protein (StAR), cytochrome P450 cholesterol side-chain lyase (P450scc) and 17-beta-hydroxysteroid dehydrogenase (17β-HSD) was measured in the testis using immunohistochemistry method.

Materials and methods

Test Material:	Glyphosate original powder
Origin:	Jinyu Chemical Co., Ltd.
Lot/Batch number:	Not reported
Purity:	95%
CAS#:	Not reported
Vehicle:	1% Carboxymethylcellulose
Test Animals:	
Species	Rat
Strain	SD
Age/weight on arrival	80-120 g

Source	Beijing Weitong Lihua Experimental Animal Technology Co., Ltd.
Housing	Not reported
Acclimatisation period	Not reported
Diet	Clean maintenance feed (Beijing Keao Xieli Feed Co., Ltd.)
Water	Not reported
Environmental conditions	
Temperature	22 ± 2 °C
Humidity	50-60%
Light/dark cycle	12 hours light/dark

Animal grouping and treatment
Based on the body weight, male rats were randomly divided into four groups: control group (1% carboxymethyl cellulose), low dose group (50 mg/kg glyphosate in 1% carboxymethyl cellulose), middle dose group (200 mg/kg glyphosate in 1% carboxymethyl cellulose), high dose group (800 mg/kg glyphosate in 1% carboxymethyl cellulose). At the 4th week, 8th week and 12th week, a number of animals were anaesthetized with 10% chloral hydrate. The blood samples were collected from the abdominal aortic to isolate the serum and the testicular tissue was taken for protein expression level detection and pathological histology examination.

The determination of serum hormone level
The FSH, LH, T and E2 levels were tested according to the kit instructions, using competitive radioimmunoassay (RIA). The standard curve and the mathematical model of RIA were used to calculate the content of the substance to be tested in the sample.

Detection of testosterone synthesis-related protein expression level
The expression levels of 17β-HSD, 3β-HSD, StAR and P450scc were detected by SP method (streptavidin-peroxidase binding method). Immunohistochemical results were determined by nine-point method (total score = staining intensity × staining area). The staining intensity score 0, 1, 2, 3 was nil, mild, moderate and severe, respectively. The staining area score 0, 1, 2, 3 was positive cell ratio less than 5%, 5%~25%, 25%~50%, more than 50%, respectively.

Statistical analysis
Mean ± standard deviation was calculated. SPSS 17.0 software was used to analyse the variance of single-factor variance analysis and factorial design, and to compare the exposed group and control group. Statistical significance is represented by $p < 0.05$.

Results

The effect of glyphosate on testicular viscera ratio in rats
The effect of glyphosate on testicular viscera ratio in rats increased with the increase of exposure dose, reaching statistical significance in the high dose group at the 12th week (see Table 1).

Table 1. Effect of glyphosate on the ratio of testis to the body weight of rats (mean ± SD, n = 10)

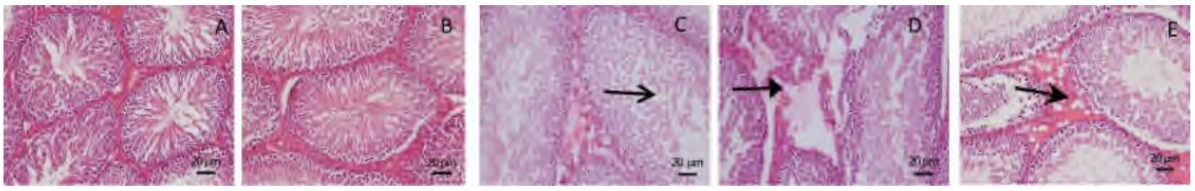
Dose group	Four weeks	Eight weeks	Twelve weeks
Control group	0.83±0.08	0.77±0.08	0.66±0.04
Low dose group	0.82±0.14	0.76±0.11	0.69±0.07
Middle dose group	0.87±0.10	0.74±0.04	0.71±0.05
High dose group	0.90±0.04	0.72±0.09	0.75±0.07*

Note: * $P < 0.05$, compared with the control group, statistically significant.

Effect of glyphosate on testicular morphology in rats
After 12 weeks of exposure, the basement membrane of testicular spermatogenic tubules in control group and low dose group was intact, the cells were arranged regularly at all levels, there was no necrosis and exfoliation, and the stroma structure was complete (Fig. 1A and B). In the middle dose group, the basement membrane of spermatogenic tubule was complete, but the arrangement of spermatogenic cells at all levels was disordered and loose, some of the cells fell off into the lumen, and the structure of stroma was loose (Fig. 1C). In the high dose group, the basement membrane of spermatogenic tubule was broken, the spermatogenic cells at all levels were

arranged in disorder, loosened and even falling off into the lumen, accompanied by the destruction of the structure of part of the stroma and the oedema of testicular tissue (Fig. 1D, E).

Figure 1. Pathological changes of testis. Note: A = control group, B = low dose group, C = middle dose group, D+E = high dose group. Arrows show (in the same order): permutation and loosening of spermatogenic cells, structural damage of interstitial area, and oedema.



Effect of glyphosate on sex hormone levels in rats

Analysis of the design variance showed that the exposure dose of glyphosate had a significant effect on the expression level of T, and also on the expression level of FSH. Time had a significant effect on T content, but did not have a significant effect on FSH, and there was no interaction between dose and time. The single factor analysis showed that the FSH and T levels in serum decreased with the increase of dose and time. The levels of T in the high-dose group were significantly lower than those in the control group at the 8^{th} and 12^{th} weeks, and the FSH levels in the high-dose group at the 12^{th} week were significantly lower than those in the control group. There was no statistical difference in LH and E2 levels in the infected group compared with the control group, and the results are shown in Table 2.

Table 2. Changes of sex hormones in rats after glyphosate exposure (mean ± SD, n = 10)

Dose group	Time	Follicule-stimulating hormone (FSH) /(mIU•mL^{-1})	Luteinizing hormone (LH) /(mIU•mL^{-1})	Testosterone (T) /(ng•mL^{-1})	Estradiol (E2) /(pg•mL^{-1})
Control group	4 weeks	1.69±0.33	2.08±0.98	1.81±0.65	15.47±4.54
	8 weeks	1.71±0.45	2.54±0.86	4.12±0.67	13.31±4.55
	12 weeks	1.66±0.39	1.55±0.38	3.38±0.37	13.90±9.15
Low dose group	4 weeks	1.64±0.66	2.22±0.77	1.61±0.38	13.64±5.28
	8 weeks	1.69±0.26	2.28±1.00	3.64±0.68	13.29±5.43
	12 weeks	1.65±0.18	2.24±0.34	3.12±0.33	11.54±4.39
Middle dose group	4 weeks	1.60±0.47	2.36±0.84	1.47±0.96	12.70±3.02
	8 weeks	1.68±0.36	2.18±0.72	3.63±0.39	13.23±3.08
	12 weeks	1.63±0.50	2.10±0.72	2.51±0.38*	11.53±3.45
High dose group	4 weeks	1.52±0.36	2.48±0.34	1.42±0.82	12.08±2.97
	8 weeks	1.43±0.38	2.17±0.94	3.27±0.57*	13.12±4.59
	12 weeks	1.23±0.28*	1.98±0.80	2.29±0.44*	11.50±5.20

Note: * $P < 0.05$, compared with the control group, statistically significant.

Effect of glyphosate on testosterone synthesis-related proteins

Immunohistochemical experiments showed that the expression levels of StAR and P450scc protein in the middle and high dose groups decreased gradually with increasing dose, and the expression levels of 17β-HSD and 3β-HSD proteins did not change significantly. The experimental results are shown in Fig. 2, Fig. 3 and Table 3.

Figure 2. Effect of glyphosate on P450scc expression. Note: A = control group, B = low dose group, C = middle dose, D = high dose group.

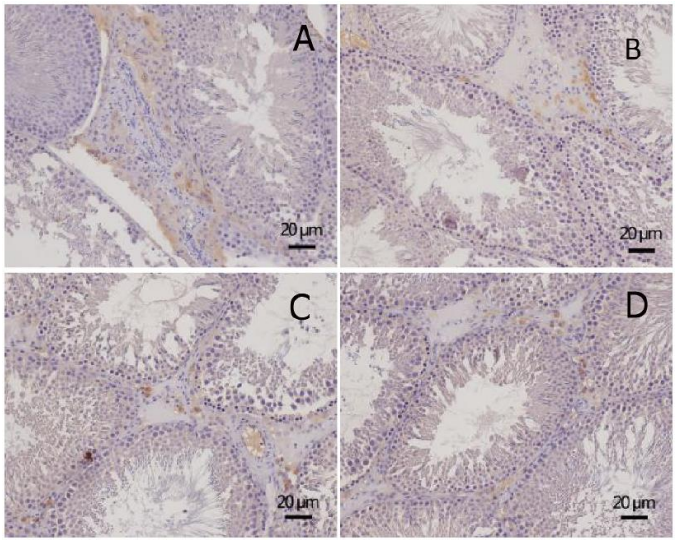


Figure 3. Effect of glyphosate on StAR expression. Note: E = control group, F = low dose group, G = middle dose group, H = high dose group.

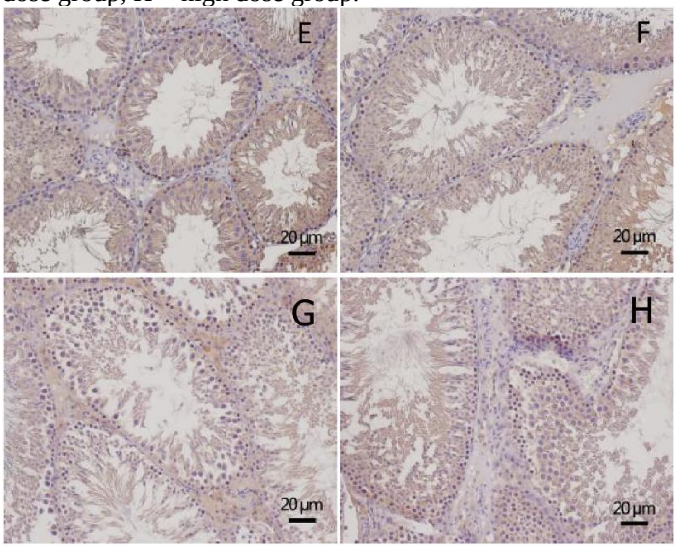


Table 3. Effects of glyphosate on testosterone synthesis-related proteins (mean ± SD, n = 10)

Dose group	StAR	P450scc	3β-HSD	17β-HSD
Control group	6.00±1.58	5.80±1.10	3.60±1.67	1.40±0.90
Low dose group	5.20±1.10	4.80±1.79	3.20±1.10	1.40±0.90
Middle dose group	2.40±0.89*	3.00±1.00*	2.80±1.10	1.80±0.45
High dose group	1.60±0.89*	2.80±1.10*	2.80±1.10	1.20±0.84

Note: * $P < 0.05$, compared with the control group, statistically significant.

Conclusion

The pathological results showed that the spermatogenic tubules, spermatogenic cells and stroma structure of testes in high dose glyphosate group were damaged to varying degrees, in which the damage of interstitial glands would affect T synthesis and secretion, leading to the decrease of T synthesis ability of rats. The testicular viscera ratio increased after exposure, which was consistent with the result of testicular oedema observed by pathology.

In this experiment, the decrease of FSH and T levels in serum of rats showed that glyphosate could interfere with the synthesis and secretion of FSH and affect the synthesis of androgen, resulting in the decrease of T level. The raw material of T synthesis is cholesterol, and the transmembrane transport of cholesterol from the outer mitochondrial membrane to the intima is a rate-limiting step for T synthesis. StAR (steroidogenic acute regulatory protein) is a cholesterol transporter that plays a very important role in T synthesis. The results of this experiment showed that with the increase of the toxic dose, the expression level of StAR decreased significantly, resulting in the obstruction and restriction of the transmembrane transport of cholesterol from the outer membrane of mitochondria to the intima, reflecting T synthesis. P450scc is cytochrome p450 cholesterol sidechain cleavage enzyme. Cholesterol synthesizes pregnenolone under the catalysis of P450scc. Pregnenolone is an important precursor for the synthesis of T. The inhibition of P450scc affects the formation of pregnenolone in cholesterol mitochondria, resulting in a decrease in pregnenolone content. Even though StAR transports cholesterol in large quantities into mitochondria, T synthesis can also be reduced due to a decrease in p450scc level. The results of this experiment showed that the expression level of p450scc gradually decreased with the increase of the toxic dose, which reduced the synthesis of pregnenolone, resulting in the decrease of T synthesis. Pregnenolone produces androdenone under the action of enzymes such as 3β-HSD and then produces T by 17β-HSD and other enzymes. The results of this experiment showed that the expression levels of 3β-HSD and 17β-HSD in the infected group were not significantly different from those in the control group, indicating that glyphosate could not change T synthesis by affecting the expression levels of 3β-HSD and 17β-HSD.

In conclusion, on the one hand, glyphosate affects T synthesis and secretion by lowering FSH levels; On the other hand, by reducing the expression levels of the related proteins StAR and P450scc of T synthesis, glyphosate affects the content of cholesterol transport into the mitochondria and the process of the pregnenolone production of cholesterol, resulting in T synthesis disorders. The combined action of the two results in the decrease of the body T level after glyphosate is infected, resulting in the toxic effect on male reproduction.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: The test material was of relatively low overall purity and not analytically verified. The number of animals tested and euthanised was not reported. Incomplete reporting of treatment procedure (frequency of dosing not clarified, number of animals used and killed at the different time points not clarified), housing conditions not reported, high doses used (>5000 mg/kg bw/day). Only in a few instances was significance indicated at the 0.05 level with no historical control data or any references provided.

Assessment and conclusion by RMS:

In this study, male SD rats were exposed to different levels of glyphosate for 4, 8 or 12 weeks. Serum contents of FSH, LH, testosterone and estradiol were measured. The testis were examined for histopathology and expression levels of testosterone synthesis related proteins was determined. Glyphosate decreased the testis to body weight ratio (high dose 12 weeks) with histopathological findings. FSH and testosterone levels were decreased and expression levels of StAR and P450cc protein were decreased in the mid- and high-dose groups.

The study is considered as supplementary data only. The study is carried out with glyphosate of lower purity (although in line with the EU agreed minimum purity) but without further details (e.g. batch info). High doses were tested without justification for selection of the dose levels. Furthermore, it seems general toxicity was not considered in this study, therefore it cannot be determined whether the results described are secondary to general toxicity.
The study is reliable with restrictions because of the following reasons: the study was not conducted according to any international guideline, no GLP status, the test substance is not sufficiently characterised, no details on group sizes was included, details on methods are missing (e.g. acclimatisation period not reported, housing conditions), it is unclear if general toxicity was measured (e.g. clinical observations, body weight, food consumption), individual data missing, no historical control data provided and positive control missing.

B.6.9. MEDICAL DATA AND INFORMATION

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

Monsanto Glyphosate Manufacturing Industrial Hygiene Monitoring Data, Luling, Louisiana, USA

Industrial hygiene air monitoring data for glyphosate with workers at the Monsanto Luling, Louisiana manufacturing facility are available for the years 1981-1998 and are presented below. No such data are available from a Monsanto European manufacturing facility. Based on the measured low exposures to glyphosate in the manufacturing setting (well below the ADI) and low toxicological concern, glyphosate specific medical monitoring is not considered necessary. These data are air concentration measurements which are conservatively applied as 100% bioavailable to calculations of mean and maximum daily exposures.

Table 6.9.1-1: Particulate exposures from glyphosate technical acid operations involving wetcake, e.g., supersack or container filling operations. Values are time weighted averages.

Glyphosate Technical Dust (mg/m^3)					Mean Daily Exposure* (mg/kg bw/day)	Maximum Daily Exposure* (mg/kg bw/day)
Sample Type	**# Samples**	**Range**	**Mean**	**SD**		
All	179	0.0003-0.2594	0.00647	0.0218	0.00108	0.04323
Personal	176	0.0003-0.2549	0.00655	0.022	0.00109	0.04248
Area	3	0.0008-0.024	0.00153	0.00081	0.00026	0.00400
Operator	158	0.0008-0.2594	0.00727	0.0235	0.00121	0.00393
Maintenance	16	0.0005-0.0053	0.00206	0.00144	0.00034	0.00088
Lab	2	0.0003-0.0004	0.00035	N/A	0.00006	0.00007

* based on breathing 10 m^3 air/shift and 60 kg worker

Table 6.9.1-2: Particulate exposures from glyphosate isopropylamine salt liquid formulation involving bottling, drumming and tote filling operations. Values are time weighted averages.

Glyphosate IPA Salt- Liquid Formulations (mg/m^3)					Mean Daily Exposure** (mg/kg bw/day)	Maximum Daily Exposure** (mg/kg bw/day)
Sample Type	**# Samples**	**Range**	**Mean**	**SD**		
All	72	0.0001-0.47	0.085	0.105	0.01050	0.05804
Personal	58	0.0001-0.47	0.0251	0.106	0.00310	0.05804
Area	14	0.004-0.28	0.0932	0.105	0.01151	0.03458
Operator	54	0.0001-0.47	0.0966	0.11	0.01193	0.05804
Maintenance	4	0.0041-0.0088	0.00792	0.00187	0.00098	0.00099

** based on breathing 10 m^3 air/shift and 60 kg worker and divided by 1.3496 to convert IPA salt to technical acid

Improvements in manufacturing facility containment and ventilation systems over recent years further reduce the likelihood of operator exposures within glyphosate manufacturing facilities.

Comments by RMS:
The above text was provided by the applicant. No further comments.

B.6.9.2. Data collected on humans

Please refer to B.6.9.3 and B.6.9.4 were information available on direct observations and epidemiology is referenced. Further references and full summaries with regards to biomonitoring or other exposure information is provided at B.6.9.8.

B.6.9.3. Direct observation

The summary in this section is based on well over 30 years of experience with numerous formulations of glyphosate in a wide range of situations. The extensive use of glyphosate has encouraged clinical assessment of various interventions and has resulted in reporting of alleged associations of symptoms with exposures to glyphosate products. The clinical toxicology of glyphosate and of glyphosate-surfactant formulations have been the subject of an extensive review (Bradberry et al 2004), and a review of cases with assessment of clinical prognostic factors was more recently published (Lee et al. 2008). Summaries of the references are provided in section B.6.9.8.

GENERAL:
Glyphosate does not inhibit cholinesterase, and has no cholinergic effect. Animals do not have the shikimic acid pathway; and no direct target-mediated action in mammalian systems has been clearly identified to date (Bradberry et al. 2004). While incidental exposure in glyphosate-surfactant herbicide mixtures is common, review of available case reports (AAPCC 2003-2011) indicates that the vast majority of reported non-suicidal exposures involve skin and/or eye irritation or irritation of the respiratory tract by inhalation of spray mist, and that systemic symptoms are rare following non-suicidal exposures to glyphosate products. Based upon human experience and animal data, even those systemic symptoms reported following incidental exposure appear unlikely to be causally related to exposure (Goldstein et al. 2002).

CLASSIFICATION OF EXPOSURES:
The following clinical effects are divided into those expected following minor and significant exposures for each category based upon expected severity of systemic symptoms. The factors which determine if the exposure is minor or significant include:

- The route of exposure. Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures for purposes of the symptom descriptions below. Ingestions more than 50 ml (one mouthful if amount unknown) of a product with >10% glyphosate concentration may be significant.
- The concentration of the product. Glyphosate concentrations of less than 10% rarely if ever produce significant toxicity. Most serious illness has historically resulted from ingestion of the 41% (glyphosate IPA) concentrate. In the absence of extensive clinical experience for the 11-40% concentration range, any ingestion of greater than 50 ml of a glyphosate preparation having a greater than 10% concentration of glyphosate salts should be considered potentially significant for purposes of the symptom descriptions below.
- The intent of the exposure. Accidental ingestion rarely involves large quantities of concentrated formulations. Intentional ingestion cases may not present with a reliable history and may require observation if the amount ingested cannot be reliably determined.
- Clinical condition of the patient.
- Known or suspected co-ingestants (if any).
- Professional judgment.

ROUTE AND ORGAN SYSTEM SPECIFIC SYMPTOMS OF EXPOSURE:

DERMAL

MINOR EXPOSURES:

- Contact with skin may produce a dermatitis similar to that of detergents (Bradberry et al. 2004)
- It is expected that the severity of injury following skin exposure will be significantly decreased with a less concentrated product and with a reduced duration of contact.
- Phototoxic reactions (sunlight or ultraviolet (UV) light induced skin reactions) have been reported. This

is believed due to an antimicrobial additive (benzisothiazolone) which is present in selected residential use (i.e. non- agricultural) products containing 10% glyphosate or less (Bradberry et al. 2004). Note by RMS: However, according to an evaluation by the Scientific Committee on Consumer Safety[13] in 2012, benzisothiazolone is not considered phototoxic.

- Significant absorption through the skin does not occur
- Studies in farmers and farm family members during the machine spray application of glyphosate products indicates that farmer exposure is generally far below recommended maximal daily intakes and that urinary levels in children and spouses are largely non-detectable (limit of urinary detection 1 µg/L) (Acquavella et al. 2004). These studies do not provide a quantitative measure of dermal exposure, but are consistent with the primate data noted above.

SIGNIFICANT EXPOSURES:

- Skin exposures are not expected to cause systemic effects or serious cutaneous effects. Symptoms as noted in the minor exposure may occur.

OCULAR

MINOR EXPOSURES:

- A review of ocular exposures to US glyphosate-surfactant formulations (1513 exposures over a 5-year period), showed no permanent eye injury (Acquavella et al. 1999).
- Human eye exposures to glyphosate-based formulations have generally resulted in temporary conjunctival irritation, clearing after irrigation or in 1-2 days and permanent eye damage is said to be "most unlikely" (Bradberry 2004).
- It is expected that the severity of injury following eye exposure will be significantly decreased with a less concentrated product or with a reduced contact time.

SIGNIFICANT EXPOSURES:

- Eye exposures are not expected to cause systemic effects or serious ocular injury (Acquavella et al. 1999; Bradberry et al. et al. 2004).

SYSTEMIC EXPOSURE- INGESTION OR INHALATION

NEUROLOGIC:

MINOR EXPOSURES:

- There is no clinical or experimental evidence that glyphosate or glyphosate-surfactant formulations cause neurological symptoms or injury after exposure by any route.

SIGNIFICANT EXPOSURES:

- There have been no reports of primary convulsions after ingestion.
- One author reports most patients present with a clear sensorium unless another substance, such as alcohol, has been co-ingested or severe hypoxemia has occurred (Tominack 1989); however "moderate disorders of consciousness" have been reported within 48 hours of suicidal ingestions of the concentrate (Sawada and Nagai 1987; Sawada et al. 1988). This has occurred in patients with significant systemic illness and is not believed to be the result of reduced organ perfusion (Bradberry et al. 2004) or perhaps other factors such as metabolic disturbance, but the possibility of a direct toxicological effect cannot be excluded (Bradberry et al. 2004).
- There are three isolated case report of Parkinson's disease developing in individuals with a history of glyphosate product exposure. In one case, Parkinson's disease of relatively acute onset was diagnosed 6 months following incidental dermal exposure to a glyphosate-surfactant product (Barbosa et al. 2001). It appears that the same case was reported as part of a case series by daCosta et at (2003) [Similar list of authors on both publications, case descriptions and ages match (52 years old at diagnosis vs 54 year old with a 2 year history of Parkinsons) and the T2-weighted Axial MRI images shown appear to be identical]. The second case (Wang et al. 2011) reports the development of Parkinson's of a 44-year old woman who had been employed in a glyphosate manufacturing facility. The third case described a woman who developed transient Parkinsonism that was reportedly reversed by the administration of atropine and pralidoxime (Zheng et al 2018). In all instances, there is no evidence for causation other than a history of prior exposure. In the last case, it is notable that the patient recovered with the treatment for organophosphate exposure, which suggests a completely different aetiology as glyphosate does not require

[13] https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_099.pdf

treatment with anticholinergic agents. No other human or animal data support the contention that Parkinson's disease results from exposure to glyphosate, even following massive ingestion or prolonged exposure.

GASTROINTESTINAL:

MINOR EXPOSURES:

- Minor exposures are likely to be asymptomatic, but the patient may experience an unpleasant taste, tingling, mild self-limited nausea and vomiting.
- Self-limited diarrhoea may also occur, which is thought to be due to the surfactant.

SIGNIFICANT EXPOSURES:

- A burning sensation in the mouth and throat, salivation, oral erythema, sore throat, dysphonia, dysphagia, epigastric pain, nausea, spontaneous vomiting, abdominal pain and diarrhoea are common and may last up to a week.
- Serum amylase may be elevated; isoenzyme analysis done in a few cases identified a salivary gland origin (Tominack et al. 1989).
- In severe cases with large ingested doses, hematemesis, GI bleeding, melena and hematochezia may occur. Paralytic ileus has been reported as a rare event.
- Endoscopy has noted erosions of the pharynx and larynx, esophagitis and gastritis with mucosal oedema, erosions and haemorrhage. Transmural injury and perforation have not been noted on panendoscopy (Chang et al. 1999).
- In fatal cases, autopsy notes mucosal or transmural oedema and necrosis throughout the small bowel with erosion and haemorrhage; in the large bowel, mucosal oedema and focal haemorrhage was noted (Tominack et al. 1989).
- Clinical, autopsy and experimental evidence (Mizuyama 1987) indicate a potential for gastrointestinal damage from glyphosate components of glyphosate formulations, but the frequency of severe injury appears to be low and early endoscopy is probably not indicated (see below).

CARDIOVASCULAR:

MINOR EXPOSURES:

- Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Cardiovascular effects are not expected from minor exposures. A recent case report describes a patient who presented with syncope and a wide-complex tachycardia where the authors claim that there is a prolonged QTc while measuring a wide complex beat. This is not how a QT interval is determined, it should be derived from the measurement of a narrow complex as a prolonged QRS interval actually makes it impossible to determine the QTc. The patient, when pressed repeatedly about pesticide exposure, said that she had spilled a small amount of formulated glyphosate on her hand the previous evening. The authors attribute her syncope and arrythmia to this minor exposure. Since significant absorption through the skin does not occur (<0.2% for concentrates and <0.01% for dilute formulations; see section 5.9.9), it is inaccurate to claim that a small dermal exposure can trigger a malignant arrhythmia, furthermore, glyphosate is not cardiotoxic and this patient appears to have early and late afterdepolarisations on her EKG, suggestive of a channelopathy.(Brunetti et al, 2020)

SIGNIFICANT EXPOSURES:

- Hypotension is common after ingestions of a mouthful or more of the concentrated product (not the diluted forms) and usually responds to IV fluids and pressor amines. Shock as manifested by oliguria, anuria and hypotension which was unresponsive to fluids and pressors, ultimately resulting in death, has been reported. (Tominack et al. 1989, Bradberry et al. 2004). Transient hypertension may be noted.

UPPER RESPIRATORY:

MINOR EXPOSURES:

- Dermal, eye and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Significant upper respiratory effects are not expected from minor exposures, but minor irritation or discomfort may occur (Bradberry et al. 2004).

SIGNIFICANT EXPOSURES:

- Significant systemic exposures are not anticipated to occur via the inhalational route, see minor exposures within this subheading.

LOWER RESPIRATORY:

MINOR EXPOSURES:

- Because of the non-volatile nature of glyphosate and the surfactant, there are no vapour exposures possible. The spray equipment commonly used with the product produces particles that are non-respirable.

SIGNIFICANT EXPOSURES:

- Tachypnea, dyspnea, cough and bronchospasm including cyanosis have been seen in severe ingestions (more than a mouthful of concentrated product). These effects appear to be the result of systemic toxicity.
- Aspiration pneumonia, pulmonary oedema and respiratory failure have been seen although the exact role of aspiration has not been fully investigated.
- An isolated case report suggests the development of acute pneumonitis in a worker following his performing maintenance on non-operating spray equipment used to apply a glyphosate-surfactant formulation (Pushnoy et al. 1998). However, the registrants do not believe that a credible mechanism of exposure was documented in this case, and the occurrence of pneumonitis in this individual was more likely coincidental in nature (Goldstein et al. 1999).
- There is also a case report out of Germany in which a glyphosate-surfactant product (tallowamine or "POEA" based) was applied by knapsack spayer in a 0.5ha forestry application at the registered application rate at 25° C for approximately 3 hours. About 7 hours after application he developed chest pain with rapidly increasing severe respiratory distress and fever up to approximately 38° C. On hospital admission, radiographic changes of lungs could be demonstrated. To further assess possible causes, bronchoscopy and closed lung biopsy was performed. Histopathology revealed "toxic inflammation of the lungs" (significantly different than bacterial infection). After 7-days of drug treatments, changes in lung reversed. Six months after the incident the patient still experienced moderate respiratory complaints on exertion. In the X-ray findings lungs showed improved results, but still detectable changes. While it is possible to differentiate acute bacterial infections on histopathology (microorganisms and polymorphonuclear leucocytic inflammatory changes should be visible), characteristics of viral, mycoplasmal, or autoimmune (vasculitic, Wegoner's granulomatosis) induced pneumonitis or Bronchiolitis Obliterans with Organizing Pneumonia (BOOP, which closely mirrors the limited case information available) are not clinically distinguishable from "toxic" etiologies. Many cases occur, most being idiopathic (no identifiable cause). Agricultural aerosols are far larger than 10 microns (generally 200 microns or so in size) and not respirable to lung, and POEA is not volatile. Contrary to this isolated case, backpack applications of glyphosate-surfactant products occur regularly in forestry and in agriculture in the developing world, without known occurrence of serious lower respiratory disease.

RENAL:

MINOR EXPOSURES:

- Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Renal effects are not expected from minor exposures.

SIGNIFICANT EXPOSURES:

- Hypotension and hypovolemic shock may result in oliguria and anuria, following severe ingestions (Bradberry et al. 2004). Abrupt rises in BUN and serum creatinine may be seen.

METABOLIC:

MINOR EXPOSURES:

- Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Metabolic effects are not expected following minor exposures.

SIGNIFICANT EXPOSURES:

- Mild fever may be noted even in the absence of infection (Bradberry et al. 2004)
- Metabolic acidosis is often seen in a severely poisoned patient (Bradberry et al. 2004) and the acidosis may fail to respond to bicarbonate therapy. Although the exact cause of the acidosis is unknown, a lactic acidosis is suspected.

HEMATOLOGIC:

MINOR EXPOSURES:

- Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Haematological effects are not expected from minor exposures.

SIGNIFICANT EXPOSURES:

- Leukocytosis without evidence of bacterial infection has been noted in peripheral blood after ingestion of the concentrate (Bradberry et al. 2004).
- Hemoconcentration has been seen as a result of intravascular volume depletion (possibly indicating severe capillary fluid leakage) (Tominack et al. 1989).
- No primary toxic effects on bone marrow or formed elements have been seen to date.

HEPATIC:

MINOR EXPOSURES:

- Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Hepatic effects are not expected from minor exposures.

SIGNIFICANT EXPOSURES:

- No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin are reported (Tominack et al. 1989; Bradberry et al. 2004).
- A 2018 case report describes a patient who developed fulminant hepatic failure after opening a bottle of formulated glyphosate with his mouth and accidentally ingesting a mouthful of the product. There is not a mechanism by which a small ingestion would cause fulminant hepatic failure nor do any GLP studies demonstrate hepatotoxicity; (Khot et al. 2018)

ELECTROLYTES:

MINOR EXPOSURES:

- Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance. This degree of illness is not generally expected from a minor exposure.

SIGNIFICANT EXPOSURES:

- Electrolytes (Na, K, Cl and Ca) in the absence of renal failure generally remain normal Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance.
- POTASSIUM SALTS: While potentially toxic ingestions of all glyphosate products may result in fluid and electrolyte disturbances, particular attention to potassium may be important following ingestion of the potassium salt products. Close monitoring of serum potassium levels and/or electro-cardiographic monitoring (for peaked T-waves or rhythm disturbances) is recommended following significant ingestion of potassium salt products, particularly for high risk individuals. Individuals with the following may be at elevated risk following acute potassium exposure: known hyperkalemia, renal failure / renal dysfunction, use of potassium sparing diuretics, hypoaldosteronism, co-ingestion of other K+ containing materials, underlying heart disease, use of digoxin, digitoxin, oabain, or exposure to other cardiac glycosides. The quantity of potassium ingested from a glyphosate potassium salt product can be estimated from the weight percent of glyphosate potassium as:

  Percent K+ salt x 5.3 = mEq potassium per 100 cc of product

- Several case reports do indicate that with large ingestions of glyphosate-potassium salt concentrate solutions, clinically significant hyperkalemia may occur. Bando et al (2001) report a 65-year old female who ingested a glyphosate-potassium salt (350 ml Roundup Maxload missing from container, in addition to 250 ml of another glyphosate formulation which was not a potassium salt- but amount actually ingested unclear) in an attempt at suicide. On admission, serum potassium level was 9.3mEq/L (typical normal value < 5) with electrocardiographic changes consistent with hyperkalemia. The patient did have a concomitant acidosis (pH 7.272) which may account for some portion of the elevation in potassium (acidosis displaces intracellular potassium). The patient responded to medical management and survived.
- Kamijo et al (2012) report a 69-year old female who ingested approximately 500 ml of the same product. On arrival in the hospital, the patient had hyperkalemia (10.7 mEq/l), pulseless ventricular tachycardia, and a severe metabolic acidosis (pH 7.005, will elevate potassium). The patient required aggressive cardiopulmonary resuscitation and hemodialysis but did recover.

- Monsanto is aware of one additional inquiry (unpublished) of a similar ingestion with a dramatically elevated potassium level in which the patient was moribund when medical care was instituted. The patient could not be resuscitated. Because serum potassium levels rise rapidly following death (due to redistribution of intracellular potassium), it is not possible to know how much of the observed hyperkalemia was the result of the ingestion versus profound acidosis and post-mortem redistribution (which is partially due to acidosis).
- It should be noted that the issue of hyperkalemia is limited to cases involving the suicidal ingestion of glyphosate-potassium concentrates. Potassium is a normal component of the human diet, and potassium intake attributable to occupational glyphosate-surfactant herbicide exposure will be negligible compared to typical dietary intake. While the concentrate formulations may contain up to approximately 250 mEq of potassium per 100 ml, product diluted for use (1% glyphosate concentration) will contain about 6 mEq potassium per 100 ml. By way of reference, a medium size banana contains about 10 mEq (425 mg) of potassium.
- Finally, it should be noted that the apparently very large (>150 ml) ingestions of glyphosate-surfactant concentrates observed in these cases are well within the range isopropylamine salt products reported to produce fatalities, and that elevations in potassium concentrations are reported (probably due to acidosis) following ingestions of glyphosate IPA salt products. While the cases do suggest that potassium salt products likely contribute to the risk of hyperkalemia, it is not clear at this time that the use of potassium salts will increase the overall clinical severity and/or mortality associated with glyphosate concentrate product ingestions.

SPECIFIC DIAGNOSTIC TESTING AND PROGNOSTIC CONSIDERATIONS

Serum or other body fluid measurements of glyphosate are generally not available in a time frame useful for acute clinical diagnosis. As the management of symptoms associated with glyphosate-surfactant product ingestion is symptom-driven in any event, the lack of rapidly available concentrations of glyphosate will generally not impair clinical care. Levels may be helpful in addressing forensic issues following clinical recovery or in the event of a fatality of unclear cause.

Attention should be paid to electrolyte concentrations in individuals with significant ingestion exposures, particularly to glyphosate-potassium concentrate solutions.

Respiratory distress requiring intubation, pulmonary oedema, shock (systolic BP < 90 mmHg), altered consciousness, abnormal chest X-ray, ingestion of over 200 cc concentrate (41%), or renal failure necessitating dialysis have been associated with a higher risk of poor clinical outcomes including mortality (Lee 2008). These authors also developed a prognostic index based upon these factors. The use of prognostic criteria does not appear to add significantly to patient care. As symptom onset may be delayed, early use of such prognostic indicators may lead to an under-estimate of clinical severity.

Comments by RMS:

The above text was provided by the applicant. Refer to Volume 1 Section 2.6.9 for a summary by the RMS (under "Direct observations").

National Chemical Emergency Centre (NCEC)

Reports from the National Chemical Emergency Centre (NCEC) in EU on any emergency calls received during the timeframe of 2017-2019. The applicant provided these incident reports of the years 2017-2019 in KCA 5.9/012 and KCA 5.9/013. The RMS has provided the following summary: In the years 2017 and 2018 76 incidents were called in to the National Chemical Emergency Centre (NCEC) in the UK. The calls were logged and characterized by the emergency responders, which is summarized in Table B.6.9.3-1 below. Most calls were of low incident severity. One incident was rated high severity and involved a large release of mixed products including products containing glyphosate on a large plot of land. The incident that was rated of medium severity involved a call made by a journalist seeking comment from the company regarding a recent court ruling in the US. This caller was forwarded to a department better suited to answer his query.
Of the low severity calls most calls were enquiring medical information or first aid and some calls were enquiring about animals. Not all medical/first aid logged calls are on humans, this category also contains calls on

medical/first aid information on animals. As said, the emergency responders have categorized the calls and it seems that there is no uniform way of categorising. Most calls of low severity were on products that were ready to use, followed by dilutions, not known and concentrated formulations. The subject of the incident were mostly adult males. However, the male and female categories also include some of the animal subjects sex. Only two (possible) exposures to children were reported. Both children lacked symptoms. One child had direct contact with the product and the other had possible contact with a contaminated surface. Of the animals involved in the low severity incidents most were pets followed by farm animals and one incident of wild animals. Two juvenile hedgehogs were found dead on a treated field. Most low severity incidents occurred after normal use and involved exposure to the skin, eyes, orally, through inhalation or on clothes. These calls concerned mostly small amounts accidentally sprayed in face or eyes or spilled on the body. For one incident no exposure to the product was reported. For almost all cases the emergency responders also categorized that there was unknown information on the exposure to the product. In two cases a wound was reported. These cases included two people who accidentally injected themselves with the product meant to be injected into the plant/tree. The emergency responders concluded that most symptoms reported were not product related as they were not expected based on the safety sheet provided. In some cases the symptoms were maybe product related and only in one incident the symptoms were concluded to be product related. Symptoms reported included irritation of eyes, skin or airways. For only a few it was reported that the product was used before the incident. However, this category was not always filled in. In 16 cases the severity of the call was reported to be not severe. The calls were enquiries of many different types. Most involved calls on the possibility to re-enter the treated area either by people or animals and the possible risks associated.

Table B.6.9.3-1 Incident summary reports 2017-2018

Incident severity	**none**	**low**	**medium**	**high**
Enquiry type				
Medical/First aid	||||	||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||		
Animal	|||||	||||| |||		
Other	|		|	
Spillage/Release	|			|
Technical Queries	|||			
Emergency Number Checks	|			
Decontamination	|			
Product details				
Ready to use	||||| |	||||| ||||| ||||| ||||| ||		
Concentrated	||||	|||||		
Dilute	|||	||||| ||||| ||||| |||||		
Residue				
Not known	||	||||| |||		|
No Product	|		|	
Information on the subject of incident				
Adult	|||	||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||		
Child	|	||		
Male	||||	||||| ||||| ||||| ||||| ||||| ||		
Female	|||	||||| ||||| ||||| ||		
Animal				
Pet	||||| |	||||| |		

Farm		|||		
Wild		|		
Information on the exposure to the product				
Normal use	||||	|||| |||| |||| |||| |||| |||| |||| |||| |||| |||| ||		
Skin	|||	|||| |||| |||| |||| ||||		
Eye		|||| |||| |||| ||||		
Inhalation	||	||||		
None	||||	|	|	|
Oral	|	|||| ||||		
Clothing		|		
Unknown	|||| |||| ||||	|||| |||| |||| |||| |||| |||| |||| |||| |||| |||| |||| |	|	|
Wound		||		
Symptoms				
Product related		|		
Not product related	|||	|||| |||| |||| |		
Maybe product related	|	|||| |||| |		
Product is used before	|	|||| |||| ||		

A similar query was conducted for calls made in the year 2019. The emergency responders of the National Chemical Emergency Centre (NCEC) in the UK only recorded the enquiry type of the incidents of 2019. Additionally short summaries of the conversation were provided by the applicant. The RMS has provided the following summary: In 2019 33 calls were made of which 23 were recorded to be not severe, 8 of low severity, 1 of medium severity and 2 of high severity (see table B.6.9.3-2). Of the calls most were technical queries informing on the dilution rates, expiry date and requesting information on re-entry after treatment. Of the non-severe calls, one call was a commercial query of a person wishing to complain on the efficacy of the product and two calls were calls requesting medical/first aid information. Of these calls one was of exposure to both an adult and child who tasted the product out of curiosity. Of the low severity incidents all calls involved accidental exposure to either a person or an animal and requesting information on the possible risks. As in the incident reports of 2017-2018 there was a case reported of a person accidentally injecting themselves with the injector after application into a tree. The medium severity incident involved a person accidentally applying the product at a much higher concentration than intended. There was no risk of water contamination in this case and the subject wanted to know the possible risks towards dogs that would be in the field the following day and if the lawn could be mowed. The high severity cases both likely involved intentional overexposure to the product. In one case an open container of Roundup missing 1-2 L of the product was found next to a dead person and the fire brigade called for information on the possible inhalation risks to the emergency responders and if the cause of death could be attributed to the product. In the other case hospital staff called for specific product information on a seriously ill patient who was reported to have ingested a Roundup product.

Table B.6.9.3-2 Incident summary reports 2019

Incident severity	**none**	**low**	**medium**	**high**
Enquiry type				
Medical/First aid	||	||||| ||		||
Animal		|		
Other				

Spillage/Release				
Technical Queries	||||| ||||| ||||| ||||			
Commercial queries	|			
Emergency Number Checks				
Decontamination			|	
Product details				
Ready to use				
Concentrated				
Dilute				
Residue				
Not known				
No Product				
Information on the subject of incident				
Adult		||		
Child	|	|		
Male		||		|
Female				
Animal				
Pet		|		
Farm				
Wild				
Information on the exposure to the product				
Normal use		|||		
Skin		||		
Eye				
Inhalation		|		
None				
Oral	|	||		|
Clothing				
Unknown		|		
Wound		|		
Symptoms				
Product related				|
Not product related				
Maybe product related		|		
Product is used before		|		

Comments by RMS:
The above text was provided by the RMS. No further comments.

B.6.9.4. Epidemiological studies

The epidemiological publications found relevant and reliable were discussed in detail in section B6.5 (carcinogenicity) of the RAR.
Besides epidemiological publications related to carcinogenicity, publications were found in the literature search related to other health outcomes. For three of these studies the RMS requested a summary in order to justify the categorization as proposed by the applicant. These three studies are included here in this section.

General introduction to the epidemiological studies:
A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the scope of their conclusions regarding either pesticides in general, certain classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, others are focussed more on pesticide use in general.
One of the main difficulties is that it is not possible to distinguish between effects of the active substance glyphosate and its co-formulants since humans are always exposed to plant protection products and their residues, but hardly ever to the active substance alone. Furthermore, as humans are exposed to a great number of environmental chemicals, it is difficult to attribute health effects directly to exposure to glyphosate. When assessing and interpreting the relevance of the findings from epidemiological studies, an essential consideration is the exposure assessment. Any suggested association between health outcomes and possible exposure to an active substance may be speculative, if exposure cannot be confirmed and quantified.

The available epidemiological studies can be divided into different categories: case-control studies and cross-sectional studies. In short, a case-control study is a study in which the investigators select persons with a certain type of disease (‘cases’) and a control group of persons without this disease (‘controls’). Then the investigators look back in time to compare the exposure – in this case to glyphosate-based formulations – of the cases compared with controls. Another type of epidemiological study is a cross-sectional study. In this type of study, exposure information and health effects information are collected at the same time. The selection of participants for the study is independent of exposure to the test agent and health-effects characteristics. Cross-sectional studies are conducted to find associations between exposure to a specific agent and development of a health effect.
The outcome parameter is an odds ratio (OR). An OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. If the calculated OR = 1, then the exposure does not affect odds of outcome. If the OR < 1, the exposure is associated with a lower odds of outcome and if the OR > 1, the exposure is associated with higher odds of outcome. Together with the OR an 95% confidence interval (95%-CI) is provided. The 95%-CI is used to estimate the precision of the OR. A large 95%-CI indicates a low level of precision of the OR, whereas a small 95%-CI indicates a higher precision of the OR. It is important to note that an OR that is statistically significant does not include the value of ‘1’ in their confidence intervals. Conversely, OR confidence intervals (CIs) that include the value ‘1’ are not statistically significant.
With the design of epidemiological studies, several uncertainties should be taken into account. For example, confounding may occur. Confounding means the distortion of the association between the independent and dependent factor because a third factor is independently associated with both. A third factor might be a confounder if it is a) associated with the outcome independent of the exposure—that is, it must be an independent risk factor; and b) associated with the exposure but is not a consequence of it. A method for looking for confounding is to stratify the exposure–outcome association of interest by the third variable suspected to be a confounder[14]. The list of potential confounders should include the known risk factors for the disease of interest (e.g. family history, smoking status) and matching variables (age, sex, social-economic status). If confounding is identified, the next step is to control for or adjust for its distorting effect by using statistical methods. When assessing a study, it should be verified that potential confounding factors are appropriately identified and considered and it should be checked how it has been controlled for these potential confounders.

Moreover, other types of bias should be considered when assessing the reliability of a study. The main types of bias include selection bias, information bias (including recall bias and interviewer/observer bias) and confounding (already discussed above).

[14] Reference: https://www.cdc.gov/eis/field-epi-manual/chapters/analyze-Interpret-Data html

B.6.9.4.1. Public literature – Occupational, dietary and other risk factors for myelodysplastic syndromes in Western Greece

1. Information on the study

Data point:	CA 5.9.4
Report author	Avgerinou C. *et al*.
Report year	2017
Report title	Occupational, dietary, and other risk factors for myelodysplatic syndromes in Western Greece
Document source	Hematology (2017), Vol. 22, No. 7, pp. 419-429
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

The authors conducted a hospital-based myelodysplastic syndrome (MDS) case–control study in two public hospitals in Patras, Greece. A total of 228 individuals (126 cases, 102 controls) were recruited in this study. MDS cases were recruited from the two participating hospitals and controls were recruited from among elective surgery cataract patients at one of the participating hospitals. Participants were interviewed based on a questionnaire regarding demographics, occupational exposures, smoking, alcohol consumption, dietary, and domestic factors. Interviewers were physicians who were not blinded to the case or control status of study participants. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Univariate analysis showed that risk of MDS was associated with a family history of hematologic malignancy or solid tumour, exposure to pesticides, insecticides, herbicides, increased weekly intake of meat and eggs, and increased alcohol intake, whereas fruit intake had a protective effect. Analysis by pesticide ingredient showed a weak association of exposure to paraquat and glyphosate with the occurrence of MDS. Multivariate analysis showed that independent risk factors for the manifestation of MDS were family history of solid tumour (OR = 2.47, 95% CI 1.32-4.65), meat intake for ≥5 days/week (OR = 2.67, 95% CI 1.05-6.80) and exposure to pesticides (OR = 3.25, 95% CI 1.73-6.11). The authors concluded that exposure to pesticides is a major risk factor for MDS in Western Greece. Family history of solid tumour and increased meat intake were also considered to play a role in the pathogenesis of MDS.

Materials and methods

Cases were prevalent MDS patients who attended the Hematology outpatient clinic at the University Hospital of Patras and at 'St Andrew' General Hospital of Patras in Western Greece. Only patients with a confirmed diagnosis of MDS, according to FAB classification after bone marrow examination were included in the study. Controls consisted of patients admitted to the Ophthalmology Department of the University Hospital of Patras for elective cataract surgery. Cases were frequency-matched by age and sex. Participation rates for cases and controls were not provided.

Hospital physicians interviewed cases and controls using the same questionnaire. Information was collected on demographic factors, family history of cancer in first degree relatives (solid tumours and hematologic malignancies), agricultural activities including use of pesticides and other chemicals, occupational exposures according to a list of exposures, diet, smoking, alcohol intake, hobbies, and diagnostic radiation exposure. Interviewers were only able to identify exposure to specific pesticides for 81% of subjects (185/228). In this

regard, the breakdown for cases and controls was not given. Interviewers were not blinded to the case or control status of study participants.

The authors used logistic regression to calculate ORs and 95% CIs as the measure of association between MDS and potential risk factors.

Results

Cases and controls were similar with respect to age (mean age 75 for both cases and controls) and sex (cases 71% male, controls 68% male). Cases were somewhat more likely to report farming as a primary (48% cases, 30% controls) or secondary (17% cases, 25% controls) occupation (overall cases 66%, controls 56%).

There were a number of significantly elevated ORs in univariate analyses including family history of a hematologic malignancy (OR = 3.56, 95% CI 1.15-11.02), family history of solid tumour (OR = 2.46, 95% CI 1.39-4.36), pesticide exposure (OR = 2.47, 95% CI 1.44-4.24), insecticide exposure (OR = 3.34, 95% CI 1.14-4.51), and paraquat use (OR = 4.90, 95% CI 1.05-22.75). Glyphosate use was associated with an elevated univariate OR of 2.57 (95% CI 0.96-6.84).

A number of dietary factors were also significantly associated with MDS in univariate analyses including meat and egg consumption. Smoking was not associated with MDS, though alcohol consumption was associated (OR = 2.06, 95% CI 1.13-4.11).

In multivariate analyses that included the frequency matching variables age and sex and all variables, where the forward selection p value was <0.10, elevated ORs were found for family history of a solid tumour (OR = 2.47, 95% CI 1.32-4.65), meat consumption 5 or more days per week (OR = 2.67, 95% CI 1.05-6.80), and exposure to pesticides (OR -3.25, 95% CI 1.73-6.11). Analyses were not presented for specific pesticides.

The authors also provided a univariate analysis for comorbidities. A number of comorbidities were negatively associated with MDS including history of osteoarthritis (OR = 0.20, 95% CI 0.09-0.46), allergy (OR = 0.20, 95% CI 0.08-0.54) and hypertension (OR = 0.44, 95% CI 0.24-0.77).

Conclusion

The authors concluded that MDS was associated with a family history of cancer that implies the importance of genetic predisposition. They also concluded that exposure to pesticides (particularly herbicides and insecticides) was associated with MDS. They were more equivocal about their findings for meat consumption.

The authors noted a number of limitations of their study. They specified recall bias and non-blinding of interviewers as concerns.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: A case-control study with non-blind interviewers results in both potential recall bias and interviewer bias. This publication is considered unreliable.

Further points for clarification:
There are a number of major limitations of this study that make the results for glyphosate unreliable. These limitations are those typical of case controls: selection bias, information bias, and uncontrolled confounding bias.

Consider selection bias first. An essential feature for a valid case control study is that the controls are representative of the population that gave rise to the cases (Rothman KJ, Greenland S, Lash T, eds., 2008). Representativeness is necessary to be able to compare validly the exposure frequency for cases with the exposure frequency in the cases' source population – the theoretical basis for the calculation of a valid exposure OR. The likelihood of selection bias is generally considered high when cases are much more likely than controls to participate in a study. That cannot be directly assessed for this study because the authors did not report information on participation for cases and controls. The likelihood of selection bias is also generally considered high when controls are selected from a restricted population. In this study, controls were selected from among surgical patients in one department from one of the two hospitals that participated in the study. That is a very

restricted control population, undoubtedly selected for convenience. Thus, it is questionable whether the exposure frequency reported by this very restricted population can be assumed to be representative of that for the population that gave rise to the cases. Accordingly, the OR will be biased to the extent that the control exposure frequency differs from the exposure frequency of the cases' source population.

A second category of likely bias in this case control study is recall bias. Recall bias is a type of information bias. Information bias refers to non-comparability of the data collected for the groups to be compared. Recall bias specifically refers to the possibility that cancer cases tend to be more likely to remember or report exposures than are controls who have not been diagnosed with cancer. This enhanced recall or reporting is thought to result from the natural self-examination by cases, but not controls, about what might have caused their grievous illness. Recall bias tends to produce spurious positive associations between exposures and diseases. Another type of information bias is interviewer bias. In this study, interviewers were aware of the case or control status of interviewees. It is standard practice to blind interviewers in case control studies due to the widespread appreciation that the collection of risk factor information can be biased by knowledge of case status. This is such a fundamental practice in case control studies that it is puzzling why the investigators did not take appropriate steps to blind interviewers.

A third area of potential concern is uncontrolled confounding. The only analyses available for specific pesticides like glyphosate were univariate analyses, without control for potential confounding factors. One cannot take univariate analyses at face value because many exposures are correlated with other risk factors. Consider, for example, the univariate findings of increased risk for family history of a hematologic malignancy (OR = 3.56, 95% CI 1.15-11.02) and family history of solid tumour (OR = 2.46, 95% CI 1.39-4.36). In multivariate analyses, the OR for family history of a solid tumour was essentially unchanged (OR = 2.47, 95% CI 1.32-4.65), but family history of a hematologic malignancy did not meet the forward selection criterion of $p < 0.10$. This illustrates appreciable confounding of the result for family history of a hematologic malignancy in the univariate analysis. While it would have been eminently logical that a family history of hematologic cancer would be predictive of MDS, the univariate result that seemed supportive of this view was confounded and unreliable. Likewise, one needs to be concerned that the univariate analysis OR for glyphosate (OR = 2.57, 95% CI 0.96-6.84) was confounded. All the other limitations aside, for this study to be informative for glyphosate, one would need to know the OR for glyphosate based on a proper multivariate analysis.

Overall, the quality of this study is very poor. This study is uninformative for glyphosate by virtue of having a very restricted control population, failing to blind interviewers, potential recall bias, and a very limited multivariate analysis for specific pesticides.

References

Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. 3rd ed. Philadelphia (PA): Wolters Kluwer/Lippincott, Williams & Wilkins, chapter 8, p 112.

Assessment and conclusion by RMS:

In this study the role of various occupational, environmental, dietary and lifestyle factors in the pathogenesis of myelodysplastic syndromes (MDS) were investigated in patients from Western Greece. A total of 228 subjects participated in the study (126 cases and 102 controls), who were interviewed about among other factors their exposure to agricultural chemicals and were asked to recall brand names of the products if they had been exposed. There is no time frame specified in the study, therefore, it is assumed that the participants were asked to recall if they were ever exposed to any of these potential risk factors. In the study an increased risk of MDS in subjects exposed to glyphosate (Roundup) was reported, although not statistically significant. Exposure to pesticides was significantly associated with the risk of MDS in a multivariate analysis.

The study is considered as unreliable by the RMS. When participants reported exposure to pesticides, they were asked to recall brand names of the products they had been exposed to, therefore, effects caused by co-formulants cannot be excluded, nor can the effects of recall bias be excluded. This was reported as one of the limitations of the present study together with the non-blind interviewers. In addition, the sample size of the study (126 cases and 102 controls) is considered to be too small. Overall, the major drawback in this study is a selection bias.

B.6.9.4.2. Public literature – Residential exposure to agricultural chemicals and premature mortality by Parkinson's disease in Washington State

1. Information on the study

Data point:	CA 5.9.4
Report author	Caballero M. *et al*.
Report year	2018
Report title	Estimated residential exposure to agricultural chemicals and premature mortality by Parkinson's disease in Washington State
Document source	International Journal of Environmental Research and Public Health (2018), Vol. 15, No. 12, pp. 2885
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

The aim of this study was to examine the relationship between assumed residential exposure to agricultural chemical application and premature mortality from Parkinson's disease (PD) in Washington State. Mortality records for Washington State for 2011-2015 were geocoded using residential addresses and classified as having exposure to agricultural land-use within 1000 meters. Generalized linear models were used to explore the association between land-use associated with agricultural chemical application and premature mortality from PD. Individuals exposed to land-use associated with glyphosate had 33% higher odds of premature mortality than those that were not exposed (Odds Ratio [OR] = 1.33, 95% confidence intervals [CI] = 1.06-1.67). Exposure to cropland associated with all pesticide application (OR = 1.19, 95% CI = 0.98-1.44) or Paraquat application (OR = 1.22, 95% CI = 0.99-1.51) was not significantly associated with premature mortality from PD, but the effect size was in the hypothesized direction. No significant associations were observed between exposure to Atrazine (OR = 1.21, 95% CI = 0.84-1.74) or Diazinon (OR = 1.07, 95% CI = 0.85-1.34), and premature mortality from PD.

Materials and methods

Data sources

Registered deaths for the years 2011-2015 were obtained from the Washington State Department of Health, Center for Health Statistics. The data file provided information regarding the individuals' sex, race, education, marital status, occupation, residential latitude and longitude, underlying cause of death, and associated International Classification of Diseases [ICD-10] codes. Data were queried to include those whose deaths were classified as a direct or underlying cause of PD [ICD-10 G20]. Agricultural chemicals were chosen based on their prevalence in the State of Washington, their geographic range of application, and their presence in previous research in correlation to neurodegenerative symptoms such as PD.

Measures

Outcome variable

The outcome of interest was premature mortality, defined as being less than 75 years of age at the time of death (yes, no).

Exposure variables

Exposure to agricultural pesticides was estimated using spatial analyses and a crop-exposure matrix that was applied to agricultural land around participants' residential addresses (drift exposure), well locations (potentially hazardous well water consumption), and occupational exposure classifications from vital statistics (occupational exposure). All analyses were conducted with ESRI ArcGIS, v.10.6.

Cropland data for the State of Washington from 2011–2015 were reclassified to align with the NAWQA crop application groups into six categories of (a) alfalfa, (b) corn, (c) orchards and grapes, (d) pasture and hay, (e) soybeans, and (f) wheat. Reclassified cropland data for the five years of interest were summed to create a single layer of agricultural land use within the given period for each type of cropland, yielding a total of six cropland layers.

To estimate the exposure to pesticide application, the following spatial procedure was conducted. Reclassified cropland data were converted from a raster to a vector format. Crop polygons of less than 18,000 m^2 (4.4 acres) were removed because previous studies on geographic analysis of agricultural field size that have shown crop sizes smaller than this are likely be digitization errors. Cropland use that aligned with the application of an individual pesticide according to the NAWQA classifications were combined to form a unique layer that represented the likely application of each agricultural chemical, yielding four individual crop-exposure layers. Residential addresses at the time of death were geocoded using ArcGIS, buffered by 1000 meters, and intersected with reclassified crop-exposure data to represent potential exposure to agricultural chemical application (ESRI, Redlands, CA, USA).

The authors classified each address into a dichotomous exposure potential, rather than a continuous measure because of the effervescence of rotational crop pesticide application, and the likelihood of pesticide drift, measuring up to 1000 meters. Measures were repeated for each individual with the three remaining pesticides.

The listed occupation that was associated with each of the deceased was used to estimate the likelihood of occupational exposure, using the industrial codes of the Bureau of Labor Statistics, classified as 'Agriculture, Forestry, Fishing and Hunting' (NAICS 11), with occupations categorized under 'Crop Production' (NAICS 111). A dichotomous variable was developed to show deaths that were associated with agricultural occupation, and thus, elevated agricultural chemical exposure (yes, no).

Demographic variables
Demographic variables were defined as sex (male, female), race (white, non-white), marital status (two categories: married or living with domestic partner versus never married, divorced, separated, or widowed), and education (four categories: no high school diploma, high school graduate or equivalent, some college, and associate's degree and or above).

Statistical analysis
Univariate analyses included the reporting measure of central tendency and variability for continuous variables and frequency distributions, and percentages for categorical variables. Bivariate statistics included chi-square and the Mann–Whitney U to test for differences in demographic and exposure variables in the premature and non-premature groups. Multivariate analyses included generalized linear models (GLMs) with a binary logistic function to explore the association between exposure and premature mortality, controlling for covariates. Associations were presented as ORs with 95% CIs. Models were adjusted for sex, race, education, and marital status.

A sensitivity analysis was conducted by exploring the relationship between chemical exposure and mortality, with the underlying cause of stroke (ICD-10, I63) that occurred in Washington State during the same period. Stroke was used as a comparison, because the biological pathway of cerebral infarction is not induced by pesticide exposure.

Results

The characteristics of individuals who experienced premature mortality are shown in Table 1. A total of 4591 PD-related deaths between 2011 and 2015 were recorded (1.75 % of all deaths), of which 659 (14%) were assumed to be premature. For all deaths attributed to PD, 94% were non-Hispanic white, and 62% were male. The median age at the time of death was 83 for all deaths with an underlying cause of PD. Approximately, 93% of those who died prematurely were non-Hispanic white, and 71% were male. Those who died before age 75 had an average age of 71 years (versus 85 for those who died at 75 or older).

Table 6.9.4.2-1. Demographic characteristics of individuals who died from Parkinson's disease from 2011-2015.

Characteristics	Total	Premature Mortality (Age ≤ 74 Years)	Non-Premature Mortality (Age > 75 Years)	*p*-Value
	(n = 4591)	(n = 659)	(n = 3932)	
Age (median, IQR)	83 (78–88)	71 (67–72)	85 (81–89)	<0.001
Sex (no. (%))				
Female	1744	189 (28.7)	1555 (39.5)	<0.001
Male	2847	470 (71.3)	2377 (60.5)	
Race/ethnicity (no. (%))				
Other	274	45 (6.8)	229 (5.8)	0.31
Non-Hispanic white	4311	612 (92.9)	3699 (94.1)	
Unknown	6	2 (0.3)	4 (0.1)	
Education (no. (%))				
Less than High School Diploma	587	66 (10.0)	521 (13.3)	<0.001
High School Diploma or equivalent	1573	191 (29.0)	1382 (35.1)	
Some college	1025	185 (28.1)	840 (21.4)	
Associate's Degree or higher	1399	216 (32.8)	1183 (30.1)	
Unknown	7	1 (0.1)	6 (0.1)	
Marital status (no. (%))				
Married or living with domestic partner	2243	399 (60.5)	1844 (46.9)	<0.001
Never married, divorced, separated, or widowed	2348	260 (39.5)	2088 (53.1)	
Likelihood of occupational exposure (no. (%))				
Likely	112	11 (1.7)	101 (2.6)	0.17
Unlikely	4479	648 (98.3)	3831 (97.4)	
Likelihood of well-water intake (no. (%))				
Likely	295	52 (18.1)	243 (81.9)	0.10
Unlikely	4296	607 (14.2)	3689 (85.8)	

Notes: IQR = Interquartile range; no = number.

In the multivariable-adjusted models, the residential exposure to pesticide that was associated with all cropland was not significantly related to premature mortality by PD, but the OR was in the hypothesized direction (OR = 1.19, 95% CI = 0.98–1.44). The residential exposure to agricultural land use associated with glyphosate had 33% higher odds of premature mortality than those who were not exposed (OR = 1.33, 95% CI = 1.06–1.67). Exposure to cropland associated with Paraquat application was not significantly associated with premature mortality by PD, but the effect size was large and in the hypothesized direction (OR = 1.22, 95% CI = 0.99–1.51). No significant associations were observed between exposure to Atrazine (OR = 1.21, 95% CI = 0.84–1.74) or Diazinon (OR = 1.07, 95% CI = 0.85–1.34), and premature mortality by PD (Table 2).

Table 6.9.4.2-2. Unadjusted and adjusted odds ratios of premature mortality by PD from exposure to individual agricultural chemicals.

Agricultural Chemical	N	Unadjusted OR (95%CI)	*p*-Value	N	Adjusted OR (95%CI)	*p*-Value
All Pesticide						
No Exposure	3526	Reference		3516	Reference	
Exposed	1065	1.16 (0.96–1.40)	0.13	1062	1.19 (0.98–1.44)	0.09
Atrazine						
No Exposure	4357	Reference		4344	Reference	
Exposed	234	1.17 (0.82–1.67)	0.40	231	1.21 (0.84–1.74)	0.31
Diazinon						
No Exposure	3833	Reference		3821	Reference	
Exposed	758	1.06 (0.85–1.31)	0.63	757	1.07 (0.85–1.34)	0.63
Glyphosate						
No Exposure	3932	Reference		3914	Reference	
Exposed	659	1.30 (1.04–1.62)	0.02	664	1.33 (1.06–1.67)	0.01
Paraquat						
No Exposure	3780	Reference		3769	Reference	
Exposed	811	1.20 (0.98–1.48)	0.09	809	1.22 (0.99–1.51)	0.07

Notes: OR = Odds Ratio; CI = Confidence Interval.

Conclusion

The authors concluded that their analyses showed an association between proximity to land treated with specific pesticides, especially glyphosate and paraquat, and mortality from PD prior to the age of 75 (as opposed to 75 and older).

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: Unproven exposure. Uncertain temporal relationship between purported exposure and the health outcome. Appropriate design would evaluate exposure or non-exposure from Parkinson's diagnosis and compare length of survival by exposure category.

Further points for clarification:
This study does not provide reliable information relevant to the ongoing evaluation for glyphosate.

The goal of this study was to determine whether residential exposure to pesticides was associated with premature death from Parkinson's disease (PD). The exposure variable used was based on residential proximity (up to 1000 meters) of a decedent's death certificate address to land where various chemicals, including glyphosate, were used. The exposure variable has obvious limitations that makes reliance on the results impossible. First, there is the unproven assumption that residence near land on which glyphosate was applied equates to any exposure, let alone meaningful exposure. The assumption behind the exposure variable is inconsistent with glyphosate urinary biomonitoring of farmers and their families that showed minimal to no quantifiable exposure, with a limit of detection of 1 part per billion, for those living on farms, but not directly involved in the glyphosate application (see Acquavella *et al*. 2004). Thus, there is no data to support an exposure variable of living within 1000 meters of where glyphosate was applied. Second, the exposure assessment is simultaneous with time of death. There is no way to know whether exposure preceded death in a way that makes sense for the hypothesis that prior exposure may be related to premature death from PD. Lastly, and related to the previous point, it is worth noting there is no information about the subjects' residential histories other than the address listed at time of death. Residence prior to death is an important aspect of exposure potential.

One can also question the conceptualization of the outcome variable. Presumably, the hypothesis would be best studied by characterizing exposure and non-exposure from onset of Parkinson's disease. Then one could compare mortality rates for exposed and unexposed patients controlling for demographic variables, relevant co-morbidities, and other confounding factors. Whether dying before age 75 is premature mortality depends on the medical histories of individual patients and cannot be dichotomized into an informative outcome variable as the authors have done. It is noteworthy that the Agricultural Health Study found no association with glyphosate use by farmers and applicators and self-reported Parkinson's disease: RR = 1.0, 95% CI 0.6-1.7 for prevalent cases and RR = 1.1, 95% CI 0.6-2.0 for incident cases (Kamel *et al*. 2007). The fact that individuals who actually apply glyphosate did not have elevated rates of Parkinson's disease suggests that glyphosate exposure is not a cause of Parkinson's disease or advancement of the Parkinson's disease process.

References

Acquavella JF, Alexander BH, Mandel JS, et al. Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study. Environ Health Perspect 2004; 112:321-326.

Kamel F, Tanner CM, Umbach DM, et al. Pesticide Exposure and Self-reported Parkinson's disease in the Agricultural Health Study. Amer J Epidemiol 2007; 165:364-374.

Assessment and conclusion by RMS:

Remark: In addition to the information included above by the applicant, the article also described some results looking at premature mortality on an individual level, in which several clusters of premature deaths were found in highly agricultural areas.
Regarding well water consumption, the authors estimated potential well water based exposure considering the well's proximity to cropland and the plausible groundwater compromise to within 500 m of cropland. No significant results were found for well water in this study.
The applicant questioned the choices for outcome variables (premature mortality <75 years of age; early vs late

onset of PD (≤65 or >65 years of age), however, the article does provide reasonable explanations for making these choices which seem to be appropriate.

In the multivariable-adjusted models used in this study, the residential exposure to pesticide that was associated with all cropland was not significantly related to premature death by Parkinson's disease (PD), but the OR was in the hypothesized direction (OR=1.19, 95%-CI = 0.98-1.44). The residential exposure to agricultural land associated with glyphosate had a statistically significant OR for premature mortality associated with PD (OR=1.33, 95%-CI = 1.06-1.67). However the study design does have its limitations.
This study is considered as supplementary data only. No actual exposure data was available in this study, as exposures were assumed based on residential proximity at the time of death to agricultural land. It is unclear to what extend glyphosate was used and what formulations of glyphosate were applied, therefore, also possible effects from co-formulants cannot be excluded.
Furthermore, no information was available on possible previous exposures of subjects as only the current address at time of death was known and no history of for instance the kind of work conducted during their lifetime was available. Also no data on other confounding factors was available or taken into account (e.g. lifestyle factors) that could have contributed to the development of Parkinson's disease.
Based on the aforementioned limitations, the study is considered to be reliable with restrictions.

B.6.9.4.3. Public literature – Occupational exposure to pesticides, reproductive hormone levels and sperm quality in young Brazilian men

1. Information on the study

Data point:	CA 5.9.4
Report author	Cremonese C. *et al*.
Report year	2017
Report title	Occupational exposure to pesticides, reproductive hormone levels and sperm quality in young Brazilian men
Document source	Reproductive Toxicology (2017), Vol. 67, pp. 174-185
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

The authors conducted a cross-sectional study of the potential effects of exposure to pesticides and reproductive hormones, semen quality, and genital measures among young men in the South of Brazil. Participants were 99 rural and 36 urban men aged 18-23 years. Information on pesticide use was obtained through a questionnaire. Serum and semen samples were analysed for sex hormones and sperm parameters, respectively, and measurement of anogenital distance (AGD) and testis volume (TV) were performed. Associations were evaluated using multivariate linear regression. Rural men had poorer sperm morphology, higher sperm count, and lower LH levels relative to urban subjects. Lifetime use of pesticides, especially herbicides and fungicides, was associated with poorer morphology and reduced LH and prolactin, with evidence of a linear trend. Maternal farming during pregnancy was associated with larger AGD and TV. The authors concluded that chronic occupational exposure to modern pesticides might affect reproductive outcomes in young men.

Materials and methods

The authors conducted a cross-sectional study between 2012 and 2013 with a random sample of men aged 18-23 years from the agricultural population of the municipality of Farroupilha, in Rio Grande do Sul, the southernmost state of Brazil. A control group of the same age was selected from the urban area of the town.

Firstly, all rural male residents in the age group under study were identified from the list of rural households of the municipal agriculture office. Among the 180 subjects identified, a sample of 80 men was randomly selected and contacted to participate in the study. An additional sample of 30 rural males was randomly selected from the list of members of the military service for the period between 2009 and 2013. Of the 110 invited rural subjects, 3 (2.7%) refused to participate. The control group was composed of men of the same age residing in the urban area of Farroupilha, randomly selected from the same military service list. From a sample of 50 urban men contacted, 4 (8%) refused to participate in the study. Subjects that previously had a vasectomy, infertility diagnosis or confirmed paternity, endocrine or reproductive disorders such as testicular cancer, and men without physical or mental capacity to answer the questionnaire were excluded from the study (8 rural men and 5 urban men). Finally, urban residents with a history of agricultural work or pesticide use were also excluded from the study (5 individuals), leaving a final sample of 99 rural men and 36 urban men.

Questionnaire and self-reported exposure variables
Information on demographics, occupation, lifestyle, gestation and birth, agricultural work practices, pesticide use, and medical history was obtained through a structured questionnaire. Trained research staff administered the questionnaires through face-to-face interviews at the participants' residences. The following variables were used in this study: age (continuous and grouped into 18-20 and 21-23 years), years of education (continuous and categorized into ≤8, 9-11 and ≥12 years), occupation (farmer; non-farmer), employed in the last 3 months (no; yes), current smoking status (non-smoker; smoker), regular alcohol intake in the last 30 days (no; yes), practised physical activity regularly in the last 3 months (no; yes), felt stress in the last 3 months (no; yes), current weight (kg) and height (cm), maternal farming during pregnancy (no; yes), maternal smoking during pregnancy (no; yes), premature birth (no: ≥37 weeks; yes: <37 weeks), low birth weight (no: ≥2500 g; yes: <2500 and), and birth length (categorized into <50 and ≥50 cm). Body mass index (BMI) was calculated by dividing weight in kg by height in meters squared and categorized as lower than 25 kg/m^2 (eutrophic) and equal to or greater than 25 kg/m^2 (overweight or obese).

Variables related to agricultural work and pesticide use were years of agricultural work (categorized into <6 and ≥6 years), years mixing or applying pesticides (categorized into ≤1 and >1 year), frequency of mixing or applying pesticides (categorized into <5 and ≥5 days per year), season of interview and blood sample collection (low pesticide use season: from March to September; high pesticide use season: from April to August), use of full personal protective equipment (PPE) (yes; no), and current use of pesticides (all) (no; yes). Age at starting farm work was calculated by subtracting the number of years of agricultural work from the current age, and categorized into two groups: 9-14 years (i.e. normal ages of puberty in males) and ≥15 years or never farming. Participants also reported on their current and former use of specific pesticides from a list of products obtained from the Brazilian Entity for Technical Assistance and Rural Extension (EMATER), which provided the trade name of commercial formulations most commonly used in the study area. Participants were also asked to report on the use of any pesticide not included in this list. Active ingredients of commercial products were then grouped into chemical and functional classes, i.e.: fungicides, insecticides, herbicides, organophosphate (OP) insecticides, dithiocarbamate fungicides, carbamates, synthetic pyrethroids, and others chemical classes. Lifetime years of overall pesticide use and for each functional and chemical class were calculated as the difference between starting and finishing years of use, regardless of simultaneous use of different pesticides of the same class, and categorized as less than 1 year, 1-5, and 6 or more years. Variables of lifetime years of use of mancozeb, glyphosate, and paraquat were also determined and categorized into never, 1-5, and ≥6 years.

Biological samples
Blood collection and analysis
During home visits to participants, an intravenous blood sample (15 mL) was collected in heparin tube between 8:30 and 10:00 in the morning after 12 h overnight fast. Plasma and serum were separated from whole blood by centrifugation. Specimens were stored at -20 ◦C in vacutainer tubes containing EDTA.
Serum concentrations of total testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone-binding globulin (SHBG), and prolactin were measured by electrochemiluminescence immunoassay using Roche kit. Free androgen index (FAI) was calculated as the percentage molar ratio of total testosterone to SHBG levels. The ratio of testosterone to LH, which represents a measure of Leydig cell function, was calculated by dividing testosterone (ng/dL) by LH (IU/dL). Normal laboratory reference range was 249-836

ng/dL for total testosterone, 1.7-8.6 IU/L for LH, 1.5-12.4 IU/L for FSH, 13-71 nmol/L for SHBG, 4.04-15.02 ng/mL for prolactin, and 30-150% for FAI.

In addition, blood acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BChE) enzyme activities were determined. Laboratory reference cut-off values for enzymatic inhibition were ≤0.56 µmol/min/mg of protein for AChE and ≤2.29 µmol/min/mL of plasma for BChE.

Semen collection and analysis

All participants were asked to abstain from ejaculation for at least 48 h. Collection, handling, preparation, and analysis of sperm samples manually followed the standard methods established by the World Health Organization in 2010. Semen specimens were collected by masturbation and stored in polystyrene tubes. Samples were kept on a hot plate at 37 °C (90 °F) for 30 min to obtain full liquefaction and were subsequently analysed by the same embryologist.

The macroscopic parameters analysed were semen volume (mL) and pH. Microscopic parameters included: sperm concentration (million per mL) and total number of sperm (sperm concentration × semen volume); progressive sperm motility, as the percentage of motile sperm (number of motile sperm/total number of sperm); and sperm morphology, as the percentage of sperm with normal morphology.

The volume was measured by suctioning the entire sample using a pipette. Sperm pH was checked with an indicator strip presenting a range going from 7.0 to 8.0, on which an aliquot of semen was deposited and the colour obtained through comparing it with colourimetric pH calibration tape. For microscopic analysis of sperm concentration and sperm motility, a volume of 5 µL liquefied semen was deposited in the center of the Makler chamber with the aid of a micropipette. The analysis was performed under phase microscopy at 200-fold increase (ocular 2000). Sperm morphology was evaluated according to Kruger's criteria (1986). Assessment of sperm morphology was performed by the Panoptic staining method using smears of 5 µL aliquots of fresh semen with frost-ended blades. The methodology consisted of passing of the following reagents: alcohol 75 °, plunging the blade 5× and allowing it to dry, xanthene solution 0.1%, dipping 3× and allowing it to dry, thiazine 0.1%, dipping 5× and allowing it to dry. This procedure was carried out by reading through an immersion objective (ocular 1000) and sperm morphology was classified as having at least 100 sperm. The Kruger methodology uses Papanicolau stain and the laboratory responsible for sperm analysis uses slides that are stained with Panoptic stain.

According to laboratory reference values, an abnormal semen sample was defined by one of the following criteria: sperm concentration below 15×10^6 /mL, percentage of motile sperm below 32%, and percentage of sperm with normal morphology below 2%. According to a study conducted by the laboratory in patients undergoing vasectomy, the reference value for morphology was set at 2% and not 4% because the colouration used was different from the originally described by Kruger.

Genital measurements

After semen collection, an urologist specialist in reproductive medicine measured width, length, and height (cm) of both testicles and AGD (cm), using a digital calliper. AGD is the distance from the posterior aspect of the scrotum to the anal verge. Testicle volume (TV) was calculated using the formula: width × length × height (cm^3) and afterwards, the arithmetic mean of left and right TV was computed. Measurements were performed in the supine, frog-legged position with the legs abducted allowing the soles of the feet to meet.

Statistical analysis

Frequency distribution, medians, and interquartile ranges were used to describe the characteristics of the participants. The normal distribution of continuous variables was examined using the Kolmogorov-Smirnov test. Comparisons between rural and urban subjects for categorical variables were performed through chi-square and Fisher tests. Differences in distribution of hormone levels, sperm parameters, genital measures, and cholinesterase activity between groups were explored using t-test and nonparametric tests.

Association between exposure variables (i.e. urban/rural residence, agricultural work-related variables, cholinesterase activity, current and cumulative pesticide use, and gestational/birth-related variables) and male reproductive outcomes (hormone levels, sperm endpoints, AGD, and mean TV) was explored by multivariate linear regression in the pooled sample of rural and urban men, using natural-logarithm transformed (log-transformed) outcome variables, which fitted normal distributions. All models were adjusted for age, smoking status, alcohol consumption, physical activity, years of education, stress, and current BMI, which are

variables identified in the literature as potential confounders. The estimated regression coefficients (β) and 95% confidence intervals (CI) were transformed back [exp(β)] on the original scale and expressed as percentage change in dependent variable per one-unit change in exposure variable, i.e. if exposure variable changes by 1 (unit), dependent variable is expected to change by 100·β %. Further multivariate analyses were conducted restricting the sample to rural men, which may be a more homogeneous group.

Results

Rural men were younger than urban men (64% of rural men were 18-20 years old versus 39% of urban men), while urban ones were more likely to have higher education (Table 1). Most rural men were farmers at the time of the interview and had been employed the last three months. Very few participants reported current smoking. All of the participants who reported being smokers were from the rural area. One-third of both rural and urban males were overweight or obese. Rural men were more likely to drink alcohol and have physical activity regularly than urban residents. Around two-thirds of rural men were born of mothers working in agriculture during pregnancy, whereas the frequency among urban subjects was only 8%. Otherwise, gestation and birth characteristics were similar: taking urban and rural men altogether, 11% had been exposed to cigarette smoke in utero, 21% were born prematurely, 8% were born with low birth weight, and 40% had a birth length <50 cm (Table 1).

Agricultural work-related characteristics and pesticide use among men residing in the rural area are shown in Table 2. Over 60% of subjects reported working for more than 5 years as a farmer, most of them had mixed or applied pesticides for more than 1 year, with a frequency of at least 5 days per year, and half of them had started farm work at the age of 9-14 years old. More than half of the rural participants did not use full PPE regularly, and less than 20% were using pesticides at the time of the interview. Herbicides were the most commonly used type of pesticides by rural men, followed by insecticides and fungicides. Regarding chemical classes, few men reported current use of OP insecticides and dithiocarbamates, very few were using pyrethroids, and none of them was using carbamates. Over one-third of rural men had handled pesticides for 6 or more years (range: <1-14 years), with 37% having used herbicides for the same period of time, followed by fungicides (35%), and insecticides (25%). Regarding individual pesticides, more than half of the rural men reported having used mancozeb, glyphosate, or paraquat.

None of the participants showed low testosterone or androgen insufficiency (FAI <30 %) (Table 3). LH was significantly lower in rural relative to urban men, among which 14% had elevated LH. Consequently, a greater testosterone: LH ratio was observed in rural males. Prolactin was elevated in 64% of rural versus 81% of urban men. Differences in sperm parameters between groups were observed for concentration, motility, and morphology: rural men had higher sperm concentration but poorer motility and morphology. Among rural subjects, 12 and 63% had abnormal motility and morphology, respectively. None of the urban men had abnormal motility and 31% had poor morphology. Regarding genital measures, rural men had larger AGD and TV. AChE levels were significantly lower among rural residents, while BChE values did not differ between groups.

In multivariate analysis for adult exposures and reproductive hormones (Table 4), testosterone showed a significant reduction of 11% in men not using full PPE. LH levels were reduced by 19% and 16%, respectively, in rural men and those mixing or applying pesticides for more than 1 year. Use of pesticides (all), fungicides, herbicides, OP insecticides, dithiocarbamates, other chemical classes, glyphosate, and paraquat for 6 or more years was significantly associated with reduced LH by 17-37%, showing significant linear trends of decrease with increasing number of years. Conversely, no association was observed between adult exposures and FSH. SHBG was significantly decreased by 16 and 17% in men that had used insecticides and OPs for more than 5 years, respectively. Although prolactin was universally increased, especially in urban participants, levels were 24% lower in rural men with greater number of years using pesticides, herbicides, and other chemical classes, with evidence of linear trends. Otherwise, prolactin was 31-43% higher in men sampled in the high exposure season and those reporting use of insecticides, OPs, dithiocarbamates, mancozeb, and paraquat for 1-5 years. Testosterone: LH ratio was positively associated with rural living, working as a farmer, and duration of mixing or applying pesticides, showing positive dose-response relationships with lifetime use of all pesticides, herbicides, dithiocarbamates, and other chemical classes. Cholinesterase activities were not associated with reproductive hormones (Table 4), nor was reported current use of pesticides (data not shown in the publication).

Regarding sperm quality (Table 5), morphology was inversely associated with living in the rural area, mixing or applying pesticides for more than one year and a frequency of at least 5 days per year, and current use of pesticides (all) and herbicides, with reductions of 20-29%. Furthermore, use of pesticides (all), pesticide classes,

and individual pesticides for 6 or more years was significantly associated with lowered morphology by 32-46%, showing significant linear trends in decreasing morphology with increasing cumulative use. Sperm count was 58% greater in rural relative to urban men, although it did not show significant association with pesticide use. Motility was not associated with adult pesticide exposure either. As observed for reproductive hormones, cholinesterase inhibition was not associated with sperm parameters. None of the exposure variables showed statistically significant associations with seminal volume or pH (data not shown). In regard to testicular size, TV was 31% larger in rural men, and was also increased in farmers and men mixing or applying pesticides for more than one year. Additionally, TV showed positive dose-response relationships with increasing lifetime use of pesticides (all), fungicides, herbicides, dithiocarbamates, and other chemical classes. As TV, AGD was increased by 9% (95%CI: 4-15%) in rural compared to urban men (data not shown).

Multivariate analysis for gestational and birth-related variables (Table 6) revealed a significant reduction of 21% in sperm morphology and significant increases of 5% and 16%, respectively, in AGD and TV among men born to women who worked in agriculture during pregnancy. Sperm concentration was not associated with gestational exposures, and motility only showed an inverse association with premature birth. Significant reductions of 16% and 33% in testosterone and SHBG levels, respectively, among men exposed to cigarette smoke in utero were also found. Additionally, men whose mothers smoked during pregnancy had higher FAI and lower testosterone: LH ratio. LH was reduced by 15% in males with low birth length, and an increase in FSH and a decrease in SHBG were observed among subjects born prematurely.

Results of multivariate analysis in rural men were quite similar to those of the pooled sample. Nonetheless, lifetime pesticide use was associated with decreased testosterone and SHBG in the rural sample, while associations of maternal farming during pregnancy with lower sperm morphology and higher genital measures (AGD and TV) did not remain significant. In addition, starting farm work at a younger age was not associated with any of the reproductive outcomes. Finally, although some associations with hormone levels were not statistically significant after excluding the 24 rural subjects recruited from the military service list, sensitivity analysis did not lead to substantial change in the main findings of the study.

Table 6.9.4.3-1. Sociodemographic and occupational characteristics, lifestyle, gestational and birth factors of study population.

N (%)	Total N = 135	Rural N = 99	Urban N = 36	p-value[a]
Sociodemographic and occupation				
Age (years)				
18–20	77 (57.0)	63 (63.6)	14 (38.9)	0.01
21–23	58 (43.0)	36 (36.4)	22 (61.1)	
Years of education				
≤8	12 (8.9)	12 (12.1)	0	<0.01
9–11	81 (60.0)	64 (64.6)	17 (47.2)	
≥12	42 (31.1)	23 (23.2)	19 (52.8)	
Occupation				
Non farmer	65 (48.1)	29 (29.3)	30 (100)	<0.01
Farmer	70 (51.9)	70 (70.7)	0	
Employed in the last 3 months				
No	9 (6.7)	3 (3.0)	6 (16.7)	0.01
Yes	126 (93.3)	96 (97.0)	30 (83.3)	
Lifestyle				
Current smoking status				
Non-smokers	127 (94.1)	91 (91.9)	36 (100)	0.11
Smokers	8 (5.9)	8 (8.1)	0 (0)	
Regular alcohol intake in the last 30 days				
No	54 (40.0)	32 (32.2)	22 (61.1)	<0.01
Yes	81 (60.0)	67 (67.7)	14 (38.9)	
Regular physical activity in the last 3 months				
No	53 (39.3)	34 (34.3)	19 (52.8)	0.05
Yes	82 (60.7)	65 (65.7)	17 (47.2)	
Stress in the last 3 months				
No	100 (74.1)	69 (69.7)	31 (86.1)	0.05
Yes	35 (25.9)	30 (30.3)	5 (13.9)	
Body mass index (BMI)				
Eutrophic (<25 kg/m^2)	91 (67.4)	68 (68.7)	23 (63.9)	0.60
Overweight or obese (≥25 kg/m^2)	44 (32.6)	31 (31.3)	13 (36.1)	
Gestation and birth				
Maternal occupation during pregnancy				
Non farmer	66 (48.9)	33 (33.3)	33 (91.7)	<0.01
Farmer	69 (51.1)	66 (66.7)	3 (8.3)	
Maternal smoking during pregnancy				
No	120 (88.9)	87 (87.9)	33 (91.7)	0.76
Yes	15 (11.1)	12 (12.1)	3 (8.3)	
Premature birth				
No	106 (78.5)	82 (82.8)	26 (72.2)	0.17
Yes	27 (21.5)	17 (17.2)	10 (27.8)	
Birth weight (g)				
<2500	11 (8.1)	8 (8.1)	3 (8.3)	1.00
≥2500	124 (91.9)	91 (91.9)	33 (91.7)	
Birth length (cm)				
<50	55 (40.7)	40 (40.4)	15 (41.7)	1.00
≥50	80 (59.3)	59 (59.6)	21 (58.3)	

SD: standard deviation.
[a] Chi-square or Fisher test.

Table 6.9.4.3-2. Agricultural work-related characteristics and pesticide use among young rural men (N = 99).

	N (%)
Agricultural work	
Years of agricultural work	
<6	38 (38.4)
≥6	61 (61.6)
Years mixing or applying pesticides	
≤1	20 (20.2)
>1	79 (79.8)
Days per year mixing or applying pesticides	
<5	20 (20.2)
≥5	79 (79.8)
Sampling season	
Low pesticide use season	61 (61.6)
High pesticide use season	38 (38.4)
Use of personal protective equipment (PPE)	
Yes	43 (43.4)
No	56 (56.6)
Current use of pesticides (all)	
No	81 (81.8)
Yes	18 (18.2)
Age at starting farm work	
≥15 years or never farming	48 (48.5)
9–14 years	51 (51.5)
Current pesticide use by functional class	
Fungicides	
No	90 (90.9)
Yes	9 (9.1)
Insecticides	
No	89 (89.9)
Yes	10 (10.1)
Herbicides	
No	85 (85.9)
Yes	14 (14.1)
Current pesticide use by chemical class	
OP insecticides	
No	91 (91.9)
Yes	8 (8.1)
Dithiocarbamate fungicides	
No	93 (93.9)
Yes	6 (6.1)
Carbamates	
No	99 (100)
Yes	0 (0)
Synthetic pyrethroids	
No	96 (97.0)
Yes	3 (3.0)
Other chemical classes	
No	84 (84.8)
Yes	15 (15.2)
Lifetime years of pesticide use by class	
All pesticides	
<1	18 (18.2)
1–5	43 (43.4)
≥6	38 (38.4)
Fungicides	
<1	27 (27.3)
1–5	37 (37.4)
≥6	35 (35.4)
Insecticides	
<1	46 (46.5)
1–5	28 (28.3)
≥6	25 (25.3)
Herbicides	
<1	23 (23.2)
1–5	39 (39.4)
≥6	37 (37.4)
OP insecticides	
<1	48 (48.5)
1–5	28 (28.3)
≥6	23 (23.2)
Dithiocarbamate fungicides	
<1	36 (36.4)
1–5	33 (33.3)
≥6	30 (30.3)
Other chemical classes[a]	
<1	18 (18.2)
1–5	43 (43.4)
≥6	38 (38.4)
Lifetime years of use of specific pesticides	
Mancozeb	
Never	43 (43.4)
1–5	28 (28.3)
≥6	28 (28.3)
Glyphosate	
Never	35 (35.4)
1–5	26 (26.3)
≥6	38 (38.3)
Paraquat	
Never	36 (36.4)
1–5	31 (31.3)
≥6	32 (32.3)

[a] Synthetic pyrethroids and carbamates included.

Table 6.9.4.3-3. Hormonal profile, semen parameters, genital measures, and cholinesterase activities in urban and rural young men

	Median (25th–75th percentiles)			% Reduced value[a]			% Elevated value[b]		
	Rural (N = 99)	Urban (N = 36)	p-value[c]	Rural	Urban	p-value[d]	Rural	Urban	p-value[d]
Reproductive hormones									
Testosterone (ng/dL)	542.5 (453.5–671.6)	495.8 (440.0–617.6)	0.55	0	0	1.00	5.1	2.8	1.00
LH (IU/L)	4.4 (3.4–5.7)	5.4 (4.2–7.1)	**0.01**	1.0	2.8	0.40	4.0	13.9	0.09
FSH (IU/L)	3.6 (2.3–5.4)	3.4 (2.0–5.9)	0.57	7.1	8.3	0.90	0	2.8	0.24
SHBG (nmol/L)	25.0 (21.0–34.2)	28.0 (18.9–32.9)	0.89	5.3	5.6	0.96	0	0	1.00
Prolactin (ng/mL)	18.1 (10.4–25.9)	20.6 (16.1–27.4)	**0.05**	2.1	0	0.12	63.9	80.6	0.16
FAI (%)	72.3 (62.5–86.4)	64.6 (55.8–88.9)	0.85	0	0	1.00	0.1	0.1	1.00
T:LH (ng/IU)	1263 (931–1602)	1071 (744–1212)	**0.01**	–	–	–	–	–	–
Semen parameters									
Seminal volume (mL)	2.3 (1.3–3.0)	2.0 (1.9–2.6)	0.64	–	–	–	–	–	–
pH	7.5 (7.5–7.5)	7.5 (7.5–7.5)	0.57	–	–	–	–	–	–
Concentration (x10^6/mL)	88.0 (36.0–160.0)	76.5 (28.0–114.0)	**0.04**	11.1	11.1	1.00	–	–	–
Motility (%)	56.0 (42.6–69.5)	64.6 (51.9–72.9)	**0.01**	12.1	0	0.04	–	–	–
Morphology (%)	1.0 (0.0–2.0)	2.5 (1.0–4.0)	**<0.01**	62.6	30.6	<0.01	–	–	–
Genital measures									
Anogenital distance (AGD) (cm)	1.8 (1.8–2.1)	1.8 (1.6–1.8)	**<0.01**	–	–	–	–	–	–
Testicular volume (TV) (cm^3)									
Right	27.6 (21.7–34.3)	23.2 (16.2–28.4)	**0.02**	–	–	–	–	–	–
Left	25.0 (20.0–30.0)	17.2 (16.0–24.9)	**<0.01**	–	–	–	–	–	–
Mean TV	26.3 (21.0–31.9)	19.2 (16.1–25.7)	**<0.01**						
Cholinesterase activity									
AChE (μmol/min/mg)	0.65 (0.55–0.72)	0.76 (0.70–0.82)	**<0.01**	31.3	2.8	<0.01	–	–	–
BChE (μmol/min/mL)	3.43 (2.74–4.08)	3.33 (2.81–4.02)	0.79	5.2	0	0.32	–	–	–

FAI: Free androgen index; T:LH: Testosterone to LH ratio.
Bold values show statistically significant p-values ($p < 0.05$).

[a] For reproductive hormones and sperm parameters, level below laboratory lower reference limit (testosterone: 249 ng/dL; LH: 1.7 IU/L; FSH: 1.5 IU/L; SHBG: 13 nmol/L; prolactin: 4.04 ng/mL; FAI: 30%; sperm concentration: 15×10^6/mL; motility: 32%; morphology: 2%). For AChE and BChE, frequency of inhibition.

[b] Level above laboratory upper reference limit (testosterone: 836 ng/dL; LH: 8.6 IU/L; FSH: 12.4 IU/L; SHBG: 71 nmol/L; prolactin: 15.02 ng/mL; FAI: 150%).

[c] *T*-test or non-parametric test.

[d] Chi-square or Fisher test.

Table 6.9.4.3-4. Adjusted[a] regression coefficients (95% confidence intervals) for percentage change[b] in hormone levels associated with exposure variables

Exposure variables	Testosterone	LH	FSH	SHBG	Prolactin	FAI	T:LH
Rural (ref = urban)	1.05 (0.94–1.16)	**0.81 (0.68–0.95)**	0.94 (0.72–1.21)	1.02 (0.87–1.21)	0.94 (0.74–1.17)	1.03 (0.90–1.17)	**1.30 (1.09–1.55)**
Farmer (ref = non-farmer)	1.03 (0.94–1.13)	0.87 (0.76–1.02)	0.86 (0.70–1.07)	0.99 (0.87–1.13)	1.17 (0.97–1.40)	1.04 (0.94–1.15)	**1.16 (1.01–1.33)**
Years of agricultural working ≥6 (ref = <6)	0.95 (0.87–1.05)	1.13 (0.99–1.28)	1.02 (0.83–1.27)	1.08 (0.95–1.25)	0.95 (0.79–1.15)	0.89 (0.79–1.15)	**0.83 (0.73–0.97)**
Years mixing or applying pesticides >1 (ref = <1)	1.02 (0.92–1.12)	**0.84 (0.71–0.96)**	0.84 (0.68–1.05)	0.93 (0.84–1.11)	1.02 (0.83–1.25)	1.05 (0.94–1.16)	**1.22 (1.05–1.42)**
Days/year mixing or applying pesticides ≥5 (ref = <5)	0.99 (0.90–1.09)	**1.21 (1.04–1.39)**	1.15 (0.92–1.43)	1.02 (0.87–1.17)	1.00 (0.83–1.22)	0.97 (0.87–1.08)	0.83 (0.71–1.10)
High pesticide use season (ref = low use season)	0.98 (0.88–1.07)	1.13 (0.97–1.30)	0.95 (0.76–1.19)	0.93 (0.81–1.07)	**1.43 (1.20–1.72)**	1.05 (0.94–1.16)	0.87 (0.75–1.01)
Not using PPE (ref = use of PPE)	**0.89 (0.80–0.98)**	1.02 (0.87–1.19)	1.10 (0.87–1.35)	0.95 (0.83–1.10)	0.98 (0.80–1.20)	0.94 (0.84–1.05)	0.87 (0.75–1.03)
AChE inhibited (ref = normal)	1.04 (0.94–1.15)	0.90 (0.77–1.08)	0.95 (0.73–1.23)	0.97 (0.83–1.14)	0.94 (0.76–1.17)	1.07 (0.95–1.21)	1.15 (0.97–1.36)
BChE inhibited (ref = normal)	1.07 (0.86–1.34)	0.86 (0.61–1.21)	0.95 (0.55–1.63)	1.09 (0.79–1.52)	0.91 (0.57–1.46)	0.80 (0.75–1.28)	1.25 (0.85–1.80)
Lifetime years of pesticide use (ref = <1 year)							
All pesticides							
1–5	0.95 (0.86–1.06)	0.86 (0.73–1.02)	0.93 (0.72–1.21)	0.95 (0.81–1.10)	1.03 (0.83–1.28)	1.01 (0.88–1.15)	1.10 (0.92–1.31)
≥6	0.92 (0.83–1.03)	**0.63 (0.65–0.89)**	0.90 (0.68–1.17)	0.86 (0.73–1.02)	**0.76 (0.60–0.95)**	1.06 (0.93–1.22)	**1.21 (1.02–1.46)**
p–trend	0.14	**0.002**	0.44	0.08	**0.02**	0.35	**0.03**
Fungicides							
1–5	0.95 (0.85–1.06)	0.94 (0.79–1.10)	0.95 (0.74–1.24)	0.91 (0.77–1.06)	1.25 (0.99–1.55)	1.06 (0.94–1.20)	1.03 (0.85–1.22)
≥6	0.96 (0.85–1.06)	**0.82 (0.68–0.97)**	0.94 (0.72–1.22)	0.87 (0.74–1.03)	0.89 (0.72–1.13)	1.08 (0.95–1.25)	1.16 (0.97–1.38)
p–trend	0.32	**0.03**	0.63	0.09	0.53	0.19	0.13
Insecticides							
1–5	1.02 (0.91–1.13)	1.00 (0.84–1.19)	1.01 (0.77–1.30)	0.97 (0.83–1.13)	**1.31 (1.05–1.63)**	1.05 (0.93–1.20)	1.02 (0.84–1.21)
≥6	0.92 (0.83–1.04)	0.85 (0.71–1.03)	1.05 (0.79–1.38)	**0.84 (0.71–0.99)**	0.91 (0.72–1.16)	1.07 (0.93–1.23)	1.08 (0.89–1.31)
p–trend	0.27	0.14	0.81	0.08	0.95	0.23	0.44
Herbicides							
1–5	0.97 (0.87–1.08)	0.85 (0.72–1.01)	0.91 (0.69–1.18)	0.95 (0.81–1.12)	1.15 (0.92–1.43)	1.01 (0.88–1.15)	1.13 (0.95–1.35)
≥6	0.93 (0.84–1.04)	**0.75 (0.64–0.90)**	0.94 (0.71–1.24)	0.89 (0.76–1.05)	**0.76 (0.63–0.97)**	1.04 (0.91–1.19)	**1.22 (1.02–1.46)**
p–trend	0.21	**0.002**	0.64	0.18	**0.05**	0.53	**0.03**
OP insecticides							
1–5	1.02 (0.91–1.13)	1.00 (0.84–1.19)	0.99 (0.76–1.29)	0.97 (0.83–1.14)	**1.31 (1.04–1.63)**	1.05 (0.91–1.18)	1.01 (0.85–1.21)
≥6	**0.89 (0.79–0.98)**	**0.83 (0.68–0.99)**	1.05 (0.79–1.40)	**0.83 (0.69–0.99)**	0.88 (0.68–1.12)	1.07 (0.92–1.20)	1.08 (0.88–1.31)
p–trend	0.14	**0.04**	0.75	0.06	0.86	0.28	0.48
Dithiocarbamate fungicides							
1–5	0.99 (0.88–1.10)	0.89 (0.76–1.06)	0.84 (0.65–1.08)	0.90 (0.77–1.06)	**1.39 (1.12–1.73)**	1.08 (0.96–1.22)	1.10 (0.93–1.32)
≥6	0.99 (0.89–1.12)	**0.83 (0.69–0.98)**	0.93 (0.71–1.22)	0.89 (0.76–1.07)	0.98 (0.78–1.23)	1.10 (0.97–1.26)	**1.19 (1.01–1.45)**
p–trend	0.94	**0.03**	0.48	0.19	0.69	0.10	**0.04**
Other chemical classes[c]							
1–5	0.95 (0.84–1.06)	0.86 (0.73–1.02)	0.84 (0.65–1.09)	0.94 (0.81–1.11)	1.03 (0.83–1.28)	1.01 (0.88–1.15)	1.09 (0.92–1.31)
≥6	0.92 (0.83–1.03)	**0.76 (0.63–0.90)**	0.93 (0.68–1.20)	0.86 (0.73–1.02)	**0.76 (0.60–0.95)**	1.06 (0.93–1.22)	1.21 (0.99–1.46)
p–trend	0.14	**0.002**	0.89	0.09	**0.02**	0.35	**0.03**
Mancozeb (ref = Never)							
1–5	1.02 (0.90–1.14)	0.92 (0.77–1.10)	0.99 (0.75–1.29)	0.93 (0.79–1.10)	**1.32 (1.05–1.67)**	1.09 (0.96–1.25)	1.10 (0.91–1.32)
≥6	1.01 (0.89–1.13)	0.86 (0.71–1.03)	0.92 (0.70–1.22)	0.92 (0.78–1.10)	0.95 (0.75–1.20)	1.08 (0.95–1.25)	1.17 (0.97–1.42)
p–trend	0.84	0.08	0.60	0.33	0.89	0.15	0.08
Glyphosate (ref = Never)							
1–5	1.10 (0.90–1.14)	0.97 (0.81–1.17)	0.88 (0.67–1.17)	0.99 (0.84–1.18)	1.23 (0.97–1.58)	1.02 (0.89–1.17)	1.04 (0.86–1.26)
≥6	0.96 (0.87–1.06)	**0.83 (0.70–0.96)**	0.99 (0.78–1.27)	0.90 (0.78–1.07)	0.89 (0.73–1.10)	1.05 (0.93–1.18)	1.16 (0.99–1.38)
p-trend	0.50	**0.02**	0.88	0.28	0.45	0.41	0.07
Paraquat (ref = Never)							
1–5	1.05 (0.93–1.17)	0.96 (0.81–1.16)	0.98 (0.75–1.29)	1.02 (0.86–1.20)	**1.36 (1.08–1.71)**	1.03 (0.90–1.17)	1.08 (0.89–1.30)
≥6	0.96 (0.86–1.08)	**0.83 (0.69–0.99)**	1.04 (0.79–1.37)	0.88 (0.74–1.05)	0.83 (0.66–1.04)	1.08 (0.95–1.25)	1.16 (0.97–1.40)
p–trend	0.63	**0.05**	0.80	0.20	0.35	0.22	0.11

PPE: Personal protective equipment; FAI: Free androgen index; T:LH: Testosterone to LH ratio; ref: reference category.
Bold values show statistically significant p-values ($p < 0.05$).

[a] Adjusted for age, smoking status, alcohol intake, physical activity, years of education, stress, and BMI (overweight/obese or eutrophic).

[b] An estimate of 1 equals 100%.

[c] Synthetic pyrethroids and carbamates included.

Table 6.9.4.3-5. Adjusted[a] regression coefficients (95% confidence intervals) for percentage change[b] in sperm parameters and genital measures associated with exposure variables

Exposure variables	Sperm parameters			TV
	Concentration	Motility	Morphology	
Rural (ref= urban)	**1.58 (1.01–2.48)**	0.88 (0.67–1.16)	**0.71 (0.55–0.93)**	**1.31 (1.14–1.54)**
Farmer (ref= non-farmer)	1.19 (0.83–1.73)	0.90 (0.73–1.14)	0.85 (0.68–1.07)	**1.20 (1.05–1.34)**
Years of agricultural working ≥6 (ref= <6)	0.81 (0.56–1.20)	0.99 (0.78–1.23)	1.19 (0.93–1.54)	**0.84 (0.75–0.96)**
Years mixing or applying pesticides > 1 (ref= ≤1)	1.25 (0.84–1.88)	1.01 (0.78–1.27)	**0.71 (0.56–0.90)**	**1.22 (1.07–1.39)**
Days/year mixing or applying pesticides ≥ 5 (ref= <5)	0.85 (0.58–1.27)	0.99 (0.78–1.26)	**0.80 (0.75–0.98)**	0.84 (0.73–1.01)
High pesticide use season (ref = low use season)	0.87 (0.59–1.30)	1.10 (0.87–1.40)	1.14 (0.88–1.43)	0.88 (0.78–1.01)
Nor using PPE (ref = use of PPE)	0.74 (0.50–1.11)	1.05 (0.82–1.34)	1.15 (0.88–1.46)	**0.85 (0.75–0.97)**
AChE inhibited (ref = normal)	0.77 (0.50–1.21)	0.95 (0.73–1.26)	1.03 (0.75–1.39)	1.13 (1.00–1.36)
BChE inhibited (ref = normal)	0.75 (0.29–1.91)	1.06 (0.60–1.89)	0.95 (0.50–1.79)	1.10 (0.79–1.54)
Current pesticide use (ref= non-use)				
All pesticides	0.76 (0.44–1.31)	0.92 (0.67–1.27)	**0.75 (0.52–0.99)**	1.06 (0.88–1.27)
Fungicides	0.80 (0.37–1.73)	0.67 (0.43–1.04)	0.93 (0.58–1.51)	0.92 (0.72–1.18)
Insecticides	0.73 (0.36–1.43)	1.05 (0.68–1.58)	0.73 (0.49–1.11)	0.99 (0.78–1.26)
Herbicides	0.91 (0.49–1.68)	0.88 (0.62–1.27)	**0.71 (0.50–0.98)**	1.04 (0.85–1.27)
OP insecticides	0.63 (0.29–1.32)	1.05 (0.66–1.66)	0.73 (0.46–1.17)	1.11 (0.85–1.43)
Dithiocarbamate fungicides	0.80 (0.31–2.09)	0.62 (0.99–1.05)	0.88 (0.51–1.58)	0.90 (0.67–1.22)
Other chemical classes[c]	0.95 (0.52–1.73)	0.86 (0.60–1.22)	0.76 (0.54–1.07)	1.04 (0.85–1.26)
Lifetime years of pesticide use (ref= < 1 year)				
All pesticides				
1–5	1.08 (0.68–1.71)	0.95 (0.73–1.26)	**0.75 (0.58–0.98)**	1.15 (0.99–1.34)
≥6	1.23 (0.76–1.99)	1.15 (0.87–1.55)	**0.55 (0.42–0.73)**	**1.17 (1.01–1.39)**
p-trend	0.39	0.33	**<0.001**	**0.04**
Fungicides				
1–5	0.74 (0.47–1.16)	0.94 (0.72–1.25)	0.82 (0.62–1.07)	1.15 (0.99–1.33)
≥6	0.99 (0.62–1.59)	1.10 (0.83–1.46)	**0.54 (0.41–0.73)**	**1.19 (1.03–1.40)**
p-trend	0.87	0.55	**<0.001**	**0.02**
Insecticides				
1–5	0.69 (0.44–1.10)	0.85 (0.64–1.13)	0.90 (0.68–1.21)	1.07 (0.91–1.24)
≥6	1.05 (0.64–1.72)	0.97 (0.73–1.31)	**0.60 (0.45–0.80)**	1.13 (0.96–1.34)
p-trend	0.77	0.62	**0.001**	0.12
Herbicides				
1–5	1.07 (0.76–1.68)	0.85 (0.64–1.13)	**0.76 (0.58–0.99)**	1.13 (0.97–1.32)
≥6	1.21 (0.76–1.93)	0.90 (0.69–1.20)	**0.58 (0.44–0.77)**	1.17 (1.00–1.38)
p-trend	0.44	0.45	**<0.001**	**0.04**
OP insecticides				
1–5	0.71 (0.45–1.12)	0.88 (0.66–1.16)	0.92 (0.70–1.23)	1.06 (0.89–1.25)
≥6	1.15 (0.69–1.89)	1.17 (0.86–1.58)	**0.61 (0.45–0.84)**	1.14 (0.96–1.36)
p–trend	0.99	0.55	**0.004**	0.11
Dithiocarbamate fungicides				
1–5	0.73 (0.46–1.14)	0.92 (0.69–1.21)	0.97 (0.73–1.30)	**1.17 (1.01–1.36)**
≥6	0.98 (0.61–1.60)	1.07 (0.80–1.42)	**0.61 (0.45–0.82)**	**1.19 (1.03–1.42)**
p-trend	0.75	0.79	**0.003**	**0.01**
Other chemical classes[c]				
1–5	1.08 (0.68–1.71)	0.95 (0.73–1.26)	**0.75 (0.58–0.98)**	1.16 (0.99–1.34)
≥6	1.23 (0.76–1.99)	3.15 (0.87–1.55)	**0.55 (0.41–0.73)**	1.08 (1.00–1.38)
p-trend	0.39	0.33	**<0.001**	**0.04**
Mancozeb (ref = Never)				
1–5	0.66 (0.41–1.05)	0.89 (0.67–1.19)	0.99 (0.74–1.34)	**1.19 (1.02–1.40)**
≥6	0.87 (0.54–1.42)	1.01 (0.75–1.37)	**0.66 (0.47–0.90)**	1.12 (0.95–1.32)
p-trend	0.36	0.91	**0.02**	0.08
Glyphosate (ref= Never)				
1–5	1.02 (0.63–1.68)	1.02 (0.76–1.38)	0.91 (0.68–1.23)	1.14 (0.96–1.34)
≥6	1.08 (0.70–1.67)	1.13 (0.87–1.47)	**0.68 (0.52–0.89)**	1.13 (0.97–1.30)
p-trend	0.74	0.37	**0.01**	0.09
Paraquat (ref = Never)				
1–5	0.79 (0.49–1.28)	0.90 (0.67–1.21)	0.90 (0.66–1.21)	1.09 (0.92–1.29)
≥6	1.13 (0.69–1.83)	1.12 (0.84–1.50)	**0.66 (0.48–0.92)**	1.08 (0.91–1.28)
p-trend	0.80	0.56	**0.02**	0.28

PPE: personal protective equipment; TV: testicular volume; ref: reference category.
Bold values show statistically significant p-values ($p < 0.05$).
[a] Adjusted for age, smoking status, alcohol intake, physical activity, years of education, stress, and BMI (overweight/obese or eutrophic).
[b] An estimate of 1 equals 100%.
[c] Synthetic pyrethroids and carbamates included.

Table 6.9.4.3-6. Adjusted[a] regression coefficients (95% confidence intervals) for percentage change[b] in reproductive outcomes associated with gestation and birth-related variables

Exposure variables	Reproductive hormones							Sperm parameters			Genital measures	
	Testosterone	LH	FSH	SHBG	Prolactin	FAI	T:LH	Concentration	Motility	Morphology	AGD	TV
Maternal farming	1.05 (0.96–1.14)	0.92 (0.80–1.06)	0.99 (0.91–1.04)	0.98 (0.86–1.12)	0.99 (0.83–1.20)	1.06 (0.96–1.17)	1.13 (0.98–1.30)	0.97 (0.67–1.39)	0.97 (0.77–1.21)	**0.79 (0.63–0.98)**	**1.05 (1.01–1.09)**	**1.16 (1.02–1.30)**
Maternal smoking during pregnancy	**0.84 (0.73–0.95)**	1.10 (0.88–1.38)	1.08 (0.77–1.51)	**0.67 (0.55–0.82)**	**0.72 (0.54–0.94)**	**1.22 (1.04–1.43)**	**0.76 (0.60–0.94)**	0.72 (0.39–1.30)	0.99 (0.69–1.42)	0.84 (0.58–1.23)	0.97 (0.90–1.05)	0.84 (0.68–1.02)
Preterm birth	0.90 (0.81–1.00)	1.06 (0.88–1.26)	**1.31 (1.02–1.70)**	**0.83 (0.70–0.96)**	0.99 (0.79–1.23)	1.09 (0.96–1.23)	0.85 (0.72–1.02)	0.65 (0.41–1.03)	**0.85 (0.64–0.13)**	0.92 (0.69–1.21)	0.99 (0.94–1.05)	0.91 (0.78–1.06)
Low birth weight	0.93 (0.80–1.05)	0.82 (0.64–1.06)	1.11 (0.77–1.63)	0.97 (0.77–1.22)	1.14 (0.83–1.58)	0.95 (0.79–1.15)	1.13 (0.87–1.46)	0.93 (0.48–1.80)	1.18 (0.79–1.75)	0.94 (0.56–1.57)	0.97 (0.90–1.06)	0.88 (0.71–1.12)
Birth length <50 cm	0.95 (0.87–1.04)	**0.85 (0.74–0.98)**	1.16 (0.94–1.42)	1.00 (0.88–1.14)	1.12 (0.94–1.35)	0.95 (0.85–1.05)	1.10 (0.96–1.27)	0.85 (0.59–1.22)	1.02 (0.82–1.27)	1.09 (0.84–1.35)	0.99 (0.95–1.05)	0.95 (0.84–1.07)

FAI: Free androgen index; T:LH: Testosterone to LH ratio; AGD: Anogenital distance; TV: Testicular volume.
Bold values show statistically significant p-values ($p < 0.05$).
[a] Adjusted for age, smoking status, alcohol intake, physical activity, years of education, stress, and BMI (overweight/obese or eutrophic).
[b] An estimate of 1 equals 100%.

Conclusion

The authors concluded that their results suggest that chronic occupational exposure to modern pesticides, particularly herbicides and fungicides, may adversely affect semen quality in young male farmers in the South of Brazil, potentially leading to poorer morphology. They also interpreted their data to suggest that exposure to agricultural pesticides may acutely increase prolactin and chronically alter sex hormone levels acting at the pituitary level through prolactin and LH suppression, hampering compensatory responses to testicular dysfunction. Whether pesticide exposure in the rural area under study can reduce sperm quality sufficiently to impair male fertility and affect pituitary function to produce clinical effects is unknown, but given that poor knowledge about pesticide risks and unsafe handling practices among small farmers in Brazil is common, the authors cautioned that the implications for public health and agricultural work could be considerable.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: Due to exposure/outcome temporal ambiguity and failure to control for other exposures in the evaluation of specific exposures. This publication is considered unreliable.

Further points for clarification:
This is a cross-sectional study that looked at myriad possible risk factors in relation to measures of development, hormone levels, and serm quality in Brazil. The primary limitation in interpreting this study is the cross-sectional design. That is, it is impossible to know whether the presumed hormonal and other changes occurred before, during, or after exposure to pesticides. A self-controlled pre/post-exposure design would be much more appropriate for the intended purposes of the study. Secondly, with respect to specific pesticide exposures like glyphosate, none of the analyses controlled for other pesticides or for maternal of gestational/perinatal factors that were also related to outcomes under study. So, confounding was not addressed appropriately.

The study has other limitations that would warrant consideration had the study not have been cross-sectional. The quality of the self-reported pesticide use information is unknown. There is also the unproven assumption that reported days of use can be used as a surrogate for dose in dose-response analyses. Previous biomonitoring research in the US for glyphosate, 2,4-D, and chlorpyrifos (Acquavella *et al*. 2006) and in Canada for 2,4-D and 4-chloro-2-methylphenoxy acetic acid (Arbuckle *et al*. 2002) would argue otherwise. In general, these studies showed that a generic approach to assessing the extent of exposure is likely to be highly inaccurate.

In conclusion, due to the temporal ambiguity between occupational pesticide exposures and outcomes, this study does not provide reliable evidence about reproductive effects due to glyphosate exposure.

References

Acquavella, JF, Alexander BH, Mandel J, et al. Exposure Misclassification in Epidemiologic Studies of Agricultural Pesticides: Insights from Biomonitoring Studies of Farmers. Epidemiology 2006; 17: 69-74.

Arbuckle TE, Burnett R, Cole D, et al. Predictors of herbicide exposure in farm applicators. Int Arch Occup Environ Health 2002; 75:406–414.

Assessment and conclusion by RMS:
This study is a cross-sectional study conducted in 99 rural and 36 urban men aged 18-23 years from the South of Brazil to investigate an association of occupational exposure to pesticides with reproductive hormone levels, semen quality and genital measures. The rural male residents were selected either from the list of rural households of the municipal agriculture office or from the list of members of the military service. The urban men were randomly selected from the same military service list. Information on occupational exposure to pesticides was obtained through questionnaires and hormone levels, semen quality and genital measures were obtained through respective blood collection and analysis, semen collection and analysis and genital measurements by an urologist. It was concluded in the study that chronic occupational exposure to pesticides might affect reproductive outcomes in young men. The study specifically reports a statistically significant association between glyphosate use for 6 or more years with reduced LH levels and lowered sperm morphology.

The study is considered to be reliable with restrictions. The study includes a multivariate analysis, i.e. the analysis is adjusted on several factors as alcohol, smoking, farmer/non-farmer, starting age of work, years of agricultural work, use of PPE, which is an advantage. The major limitation of this study is the sample size of 135 individuals and also the age categories are limited (18-20, 21-23). In addition, the study design does not allow to

identify effects of specific active substances. The statistically significant effects are found on grouped categories of pesticides (fungicides, etc). As such, the findings relate rather to cumulative exposure than anything else. Therefore, RMS considers this study to be reliable with restrictions.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

Any relevant information on poisoning is provided in B.6.9.7.

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

First aid measures

SKIN EXPOSURE:

- Remove all contaminated clothing and flood the skin surface with water.
- Wash the exposed skin twice with soap and water.
- A close examination of the skin may be required if pain or irritation exist after decontamination.
- All clothing that are contaminated should be laundered before they are worn again

EYE EXPOSURE:

- Remove contact lens from the affected eye(s) if appropriate.
- Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.
- A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably with an eye specialist.

INGESTION EXPOSURE:

- DILUTE PREPARATIONS (Glyphosate <10%): An ingestion of a dilute preparation of glyphosate (<10%) probably does not require treatment other than dilution with milk or water, and symptomatic care. Further gastrointestinal decontamination is not needed, even if spontaneous emesis has not occurred.
- Concentrated (>10%) preparations: Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 ml/kg in a child or 250 ml in an adult.

INHALATION EXPOSURE:

- No pulmonary treatment is necessary for occasional, accidental breathing of mist.
- Severe, acute pulmonary injury has not been reported following inhalation exposure. Individuals with respiratory distress from any cause should be relocated (if medically stable) to fresh air and receive supplemental oxygen if available.
- In the event of respiratory failure or lack of respiration, administer artificial respiration (or if pulse not detectable, cardiopulmonary resuscitation).

Therapeutic regimes

The registrants believe that the following represent general best practices for medical management of serious ingestions of glyphosate-surfactant products.

1. Establish respiration and assure adequacy of ventilation.

2. Eye exposure:
   A) Remove contact lens from the affected eye(s) if appropriate.
   B) Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.

C) A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably by an eye specialist.

3. Ingestion exposure:

A) Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 ml/kg in a child or 250 ml in an adult.

B) patient disposition:

Concentrated preparations (Glyphosate 41% or greater):

1) Any person ingesting greater than a large mouthful (50 ml in an adult, 0.5 ml/kg in a child) of a 41% or greater glyphosate concentrate product should be admitted to a hospital and observed for 24 hours.
2) Any adult ingesting greater than 100 ml of a 41% or greater glyphosate concentrate product (>1.4 ml/kg in a child) should be admitted to the intensive care unit.
3) Any suicide attempt by person ingesting a concentrated product should be evaluated for psychological status and should be admitted if necessary for observation with suicide precautions.

Concentrated preparations (Glyphosate 10%-40%):

An ingestion of concentrated glyphosate (10%-40%) will usually result in spontaneous emesis. There is limited experience with glyphosate formulations in this concentration range. In view of this limited information, the registrants currently recommend managing these ingestions in a manner similar to the management of the 41% concentrate.

4. Prevention of absorption *(This lists various methods for "Prevention of Absorption". These should NOT be construed as being in order of preference. Consult with Poison Center or medical personnel to determine the need for and preferred method for decontamination. In many instances, no intervention is required.):*

A) Gastric aspiration: If no significant spontaneous vomiting has occurred gastric aspiration may be considered. If performed soon after ingestion, gastric emptying by aspirating liquid gastric content with a lavage or standard NG tube may possibly remove some of the ingested glyphosate. The intent is to remove unabsorbed liquid by aspiration not to use lavage fluid. As absorption of liquids is likely to be relatively rapid, gastric aspiration after 1 to 2 hours is unlikely to be effective.

B) Emesis: Emesis is controversial at this time. Glyphosate/surfactant products are irritants. The registrants do not recommend the routine use of syrup of ipecac for glyphosate/ surfactant ingestions because of the risk of exacerbating the irritant effects on the GI tract.

C) Activated charcoal: There are no data to support or refute the use of activated charcoal in glyphosate/surfactant product ingestions. Low molecular weight, amphoteric compounds and detergents do not always bind well to activated charcoal. In the event of a mixed ingestion, activated charcoal may be advisable.

5. Assessment of gastro-intestinal injury:

Injury to the upper gastrointestinal tract may occur following ingestion of glyphosate concentrates. A study of upper gastrointestinal endoscopy following glyphosate–surfactant ingestions suggested that Zarger grade 2 lesions (erosions) were associated with longer hospital stay and with a higher incidence of serious complications (Chang 1999). However, no major esophageal or gastrointestinal injury was observed, and strictures have not been reported following uncomplicated glyphosate-surfactant ingestion.

Because no serious gastrointestinal injury is reported, and because the need for hospitalization and/or treatment of complications can be determined without endoscopic evaluation, the registrants recommend that endoscopy be reserved for patients with co-ingestions suggesting a need for endoscopy or for patients with signs and symptoms suggestive of more serious injury (serious oral burns, inability to handle secretions, clinical obstruction) regardless of clinical history.

6. Monitor blood pressure:

Monitor the patient closely for signs of hemodynamic instability.

7. Hypotension:

If the patient is hypotensive, administer IV fluid boluses and place in Trendelenburg position. If the patient is unresponsive to these measures, administer a vasopressor (dopamine, epinephrine, norepinephrine, phenylephrine.) if needed.

8. Monitor blood gases and obtain chest radiograph:

Consider the use of repeat blood gases and a peripheral pulse oximeter to monitor hypoxemia. Observe closely for sign of acidosis.

9. Pulmonary oedema:

Closely monitor arterial blood gases. If PO2 cannot be maintained above 50 mm Hg with inspiration of 60% oxygen by face mask or mechanical ventilation, then positive end expiratory pressure (PEEP) or continuous positive airway pressure (CPAP) may be needed. Avoid a positive fluid balance by careful administration of crystalloid solutions. Monitor fluid status through a central venous line or Swan Ganz catheter as needed.

10. Acidosis:

Correction of acidosis should be guided by blood gases, electrolytes and clinical judgment. Attention should be directed to volume status and correction of poor perfusion in mild cases. Sodium bicarbonate may be used to correct the acidosis in severe cases.

11. Hyperkalemia (from ingestion of Potassium salt formulations):

For moderate hyperkalemia (K+ of 6.0-7.0 mEq/L), administer sodium polystyrene sulfonate For more severe hyperkalemia (K+ > 7 mEq/L) or serious complications of hyperkalemia, correct metabolic or respiratory acidosis if present to allow potassium to enter the intracellular space. Additional management may include a glucose/insulin drip, intravenous sodium bicarbonate or calcium, and dialysis to remove excess potassium.

12. Monitor renal function closely:

Assure adequate urine output. Catheterize severely ill patients. Hemodialysis may be needed in the event of renal failure or electrolyte disturbances.

11. Enhanced elimination:

A) Forced diuresis: Glyphosate is excreted very well by the kidneys. Adequate urine flow will ensure the rapid elimination of glyphosate. Although elimination may perhaps be enhanced by forced diuresis, there is no clinical evidence that this is necessary, and fluid overload may precipitate pulmonary oedema.
B) Hemodialysis: Hemodialysis may be useful to correct fluid, electrolyte and metabolic disturbances in the patient with renal failure. The institution of hemodialysis solely to enhance the removal of glyphosate or other product components is not of proven benefit. Nevertheless, it is reasonable to consider the initiation of hemodialysis in the significantly ill patient who fails to respond to routine supportive management.

12. Serious exposure via inhalation is not expected:

Inhalation exposures are not expected due to the aerodynamics of droplet size from sprayers and because the product is not volatile. Monitor the patient for signs of respiratory compromise. Create an artificial airway if necessary. Check adequacy of tidal volume. Monitor the patient for respiratory distress; if a cough or dyspnea develop, evaluate the patient for respiratory irritation, bronchitis and/or pneumonia, but these are not expected.

13. Serious exposure via skin is not expected:

Significant skin exposures are not expected; however, the patient should be treated empirically if a dermal exposure is suspected. Remove all contaminated clothing and flood the skin surface with water. Wash the exposed skin twice with soap and water. A close examination of the skin may be required if pain or irritation exist after decontamination. All contaminated clothing should be laundered before wearing.

14. Laboratory:

Monitor electrolytes, especially if the patient is experiencing vomiting and diarrhea.15 Patients ingesting concentrated products based on the potassium salt of glyphosate may ingest large amounts

of potassium (see calculations above). Observe serum potassium and/or electrocardiogram carefully. Patients experiencing pulmonary symptoms or having chest radiograph changes should have arterial blood gas monitoring. A peripheral pulse oximeter and a Swan Ganz catheter may be needed.

Comments by RMS:

The above first aid measures and therapeutic regimes have been proposed by the applicant and were not evaluated by the RMS.

B.6.9.7. Expected effects of poisoning

Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment

The outcome of eye, dermal, and inhalational exposures, which are not expected to result in serious injury in any event, will not be significantly altered by delays in medical management. Similarly, minor oral exposures are symptomatically managed and unlikely to result in severe gastrointestinal symptoms. Medical management with intravenous fluids may provide some symptomatic relief in the event of dehydration, but recovery is anticipated in any event.

For serious ingestions having major electrolyte disturbances or life threatening alterations of cardiovascular performance, medical intervention may be life saving. Fortunately, as noted above, the onset of serious symptoms following ingestion is generally delayed by at least several hours, allowing for medical transport in all but the most remote or extreme circumstances. The availability (or lack) of acute field management does not appear likely to impact severity of survival of most serious ingestions.

Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion

Dermal exposure:

Skin irritation following exposure to glyphosate-only or glyphosate-surfactant materials is generally limited to topical irritation which will resolve within 3 days to 1 week following exposure. If exposure is aggravated by occluded conditions or physical abrasion, more severe skin injury with open skin injury may rarely result and may take longer to fully resolve.

Eye exposure:

Irritant symptoms generally resolve within 3-7 days of exposure. Most irritation is minor, but exposure to concentrate or the occurrence of a foreign body or of abrasions (from rubbing the eye) may result in corneal abrasion requiring topical antimicrobial therapy, often given in conjunction with topical corticosteroids and temporary eye patching to provide symptomatic relief. As noted above, a large study of (U.S.) ocular exposures to glyphosate-surfactant products demonstrated no long term eye injury.

Inhalation exposure:

Glyphosate-surfactant products generally do not contain readily volatile ingredients and thus inhalation exposure is limited to inhalation of agricultural droplets, which will deposit primarily in the upper airway. Resulting irritant symptoms will generally resolve within hours to a few days following exposure.

Ingestion:

Following minor or incidental ingestions, or ingestion of fully diluted formulations, gastrointestinal upset with nausea, vomiting, and diarrhoea may occur. Nausea and vomiting usually resolve within a few hours of ingestion. Diarrhoea may last for several days but is generally not severe. Following a major ingestion, the onset

of systemic symptoms may be delayed by several hours. Fatalities due to cardiovascular failure are generally delayed by 12 – 36 hours. For serious but non-fatal cases, primary clinical injury generally is manifest within 72 hours but secondary complications such as infection or respiratory distress syndrome may supervene. The majority of serious but surviving cases will be fully recovered within 7-10 days of ingestion. Individuals with complicated hospital courses can require a more extended and highly variable time to recover.

Comments by RMS:
The above text was provided by the applicant. No comments.

B.6.9.8. Literature data – medical data / treatment / poisoning / exposure

B.6.9.8.1. Literature study 1

Data point:	CA 5.9/001
Report author	Connolly, A. *et al.*
Report year	2018
Report title	Characterising glyphosate exposures among amenity horticulturists using multiple spot urine samples
Document No	doi.org/10.1016/j.ijheh.2018.06.007 E-ISSN: 1618-131X
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG:** Yes/Reliable with restrictions **Conclusion AGG:** The study is considered to be reliable with restrictions

Full summary of the study according to OECD format

The study aimed to characterise occupational exposures to glyphosate among amenity horticulturists through the collection and analysis of urine samples following pesticide application. The impact of work practices on personal exposure, as well as suitability of collecting multiple spot urine samples as a sampling strategy for the assessment of occupational exposure for glyphosate were also examined.

Materials and methods

Site description and study population - The monitoring campaign took place from September 2016 to September 2017. In this study 3 similar exposure groups (SEGs) were considered for glyphosate applications: (1) manual knapsack (Roundup Biactive XL, 360 g/L; Clinic Ace, 360 g/L; Roundup Biactive, 360 g/L; Pistol, 250 g/L; Roundup XL, 360 g/L), (2) pressurized handheld lance (Roundup Biactive XL, 360 g/L; Pistol, 250 g/L; Glyfos, 360 g/L; Rambo, 360 g/L; Roundup Gold, 450 g/L), (3) controlled droplet applicator (Nomix Dual, 120 g/L; Roundup XL, 360 g/L). Recruitment was completed in coordination with the OPW Health and Safety Unit. The lead researcher explained the project background and objectives and circulated project information leaflets to potential study participants. Participation in the study was voluntary. After having given informed consent the participants completed a self-administered questionnaire providing relevant personal and work related details, as well as information on their use of pesticides outside of work and dietary habits. Biomonitoring protocols were developed based on established protocols. Project ethical approval was received from the National University of Ireland, Galway Research Ethics Committee.

Urine collection - Biomonitoring exposure assessments took only place on the days workers were using glyphosate based pesticide products. The researcher observed all work tasks on site, and collected information such as SEG, personal protective equipment (PPE) worn, climatic conditions, and any activities or work duties performed between samples and the duration of these activities. Individual full urinary void spot samples were collected over the exposure assessment period. The void spot samples were kept and analysed separately. Time and date were mentioned by the participant on each sample container which was stored in a cooler box until collection by the researcher the morning following each task. A minimum of 3 spot urine samples were provided: a pre-task sample, a post-task sample taken within one hour of task completion and a sample taken on the following morning after the first morning void. Participants were also given the option to provide urine samples for all additional voids over the exposure assessment period (pre-task to the following first morning void sample). Participants provided more than the minimum required 3 spot samples for 59% (17) of the tasks sampled ranging between 3 and 7 samples per task. Of the spot urine samples provided, a peak urinary sample was identified for each task and defined as the highest urinary glyphosate concentration measured during the sampling period. Following sample collection, the researcher measured and recorded the volume of each urinary spot sample. A 20 mL aliquot of the well mixed spot urine sample was then transferred into a 20 mL Sterilin™ pot and labelled with a unique identifier number, date and time. Care was taken to avoid any contamination. All samples were stored at −18°C within 24 hours pending analysis.

Analysis of glyphosate in urine samples - Glyphosate was extracted from diluted urine samples (200 µL urine added to 800 µL deionised water) using anion exchange solid phase extraction (SPE) with 10% formic acid in methanol as the eluent. The eluent was evaporated under a stream of nitrogen and reconstituted in 100 µL of 0.1% formic acid for quantitative analysis by LC-MS/MS. Chromatographic separation was achieved on a Zorbax XDB-C8, 150×4.6 mm, 5 µm (Agilent, Stockport, UK) column with 0.1% formic acid and acetonitrile with a gradient elution as the mobile phase. The method was linear over the range of 0–20 µg/L and the limit of quantification (LOQ) was 0.5 µg/L. Creatinine was determined in all urine samples with a Pentra C400 clinical analyser using the alkaline picrate method.

Statistical analysis - Prior to analysis, all glyphosate concentration levels below the LOQ were imputed, using SAS v 9.4. Differences in urinary glyphosate concentrations between the pre-task samples versus the post-task and the peak urinary samples were both analysed using paired Student t-tests. Determinants of exposure on glyphosate urine concentrations were evaluated using Pearson's correlation coefficients and linear regression. A multivariate mixed effect model was elaborated to compare the glyphosate concentrations between post-task and following first morning void samples. In these models, worker identity was entered as a random effect to account for the presence of correlations between repeated measurements from the same individuals.

Results

Descriptive *and summary statistics of demographic and task characteristics* - 18 male and 2 female amenity horticultural workers applying pesticides as part of their work duties consented to participate in the study. Urine samples were collected for 29 work tasks involving glyphosate based pesticides. The concentration of glyphosate in 14 (48%) of the pre-task samples could have been influenced by work tasks performed in the days prior to this study and by starting the work task before giving the pre-task sample. For 6 (21%) of the pre-task samples, workers reported performing work tasks involving handling of glyphosate based products on the previous day to the measurement period. Similarly, for 10 (35%) of the pre-task samples (including 2 who were also involved with spraying the previous day), workers reported collecting, checking or handling potentially contaminated spraying equipment or other work equipment prior to providing the first sample (pre-task sample). A large proportion of workers reported using pesticides outside of their job (90%), corresponding to 27 (93%) of the 29 tasks included in this study. However, none reported using glyphosate based pesticides at home on the days before the sampling. The majority of the workers (60%) reported using pesticides at work for 6–7 months per year. 100% of the workers wore gloves, 90% a Tyvec suit and 97% RPE. However, 55% of the workers reported to reuse PPE.

Urinary glyphosate concentrations - 125 spot urinary samples were collected and analysed for 29 work tasks. Participants provided between 3 and 7 individual spot samples per exposure assessment period. Participants giving more than 3 spot urine samples over the exposure assessment period (n=17) allowed for a more accurate estimation of the urinary concentrations over time. There was no statistically significant difference in the average log transformed peak urinary concentrations where only the minimum of 3 samples were provided versus those

tasks where multiple samples were provided (Student t-test; p = 0.14). For 13 (45%) of the 29 tasks, the peak concentrations were identified within the samples that were collected in addition to the minimum required (pre-task, post-task and following first morning void) samples. Another 31% of the peak samples were identified in post-task samples, whereas 24% comprised of following first morning void samples. The geometric mean of the glyphosate concentrations measured in urine samples of the combined glyphosate SEGs were 0.68 µg/L for pre-task samples, 1.17 µg/L for post-task samples and 0.83 µg/L for following morning void samples. The geometric mean of the peak samples was 1.9 µg/L. Glyphosate concentrations were less than the LOQ in 34 (27%) urinary samples, of which 11 (38%) were pre-task samples and a further 11 (38%) were following first morning void samples. Two (7%) of the 29 work tasks had peak samples with urinary glyphosate concentrations below the LOQ, both belonging to the manual knapsack SEG.

Conclusion

This study provides information on occupational exposures to glyphosate among amenity horticulturalists and suggests that the collection and analysis of urine samples given up to 3 hours after task completion can be a suitable sampling strategy for estimating potential occupational exposures to glyphosate. The findings suggest that amenity horticulturists, largely complying with workplace exposure controls, have low levels of glyphosate exposures.

Assessment and conclusion by applicant:

In this study the exposure of amenity horticulturalists to glyphosate was assessed. Three similar exposure groups (SEGs) were considered for the application of various glyphosate based herbicides: one using a manual knapsack, one using a pressurized handheld lance and one using a controlled droplet applicator. Urine samples were taken pre-task, post-task and the morning after the task and analysed for glyphosate using and LC-MS/MS method with an LOQ of 0.5 µg/L. Glyphosate concentrations were found to be less than the LOQ in 27% of the urinary samples, of which 38% were pre-task samples and 38% were following morning void samples. Two of the 29 work tasks had peak samples with urinary glyphosate concentrations below the LOQ, both belonging to the manual knapsack SEG. The geometric means of the glyphosate concentrations measured in urine samples of the combined glyphosate SEGs were 0.68 µg/L for pre-task samples, 1.17 µg/L for post-task samples and 0.83 µg/L for following morning void samples. 100% of the workers wore gloves, 90% a Tyvec suit and 97% RPE.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because more detail could have been provided on the validation of the analytical method. Also the number of participants per exposure scenario was rather limited.

Reliability criteria of exposure studies – from the applicant

Publication: Connolly *et al.*, 2018	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	
Exposure to formulations with glyphosate combined with other a.s.	N	
Exposure to various formulations of pesticides	N	

Reliability criteria of exposure studies – from the applicant

Publication: Connolly *et al.*, 2018	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y?	Not complete.
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because more detail could have been provided on the validation of the analytical method. Also the number of participants per exposure scenario was rather limited.		

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. This study shows that the mean urine glyphosate concentration increases due to the pesticide application task, suggesting occupational exposure to the amenity horticultural workers. The peak glyphosate urine concentrations reported in this study had arithmetic mean, geometric mean, median and maximum levels of 2.5, 1.9, 1.9 and 7.4 µg/L respectively. 100% of the workers wore gloves, 90% a Tyvec suit and 97% RPE. However, 55% of workers reused PPE. Besides the small sample size and the limited information on the validation of the analytical method, the study report also lacks information on the bodyweight of the participants. Based on these limitations, the study is considered reliable with restrictions.

B.6.9.8.2. Literature study 2

Data point:	CA 5.9/002
Report author	Connolly, A. *et al.*
Report year	2019a
Report title	Exploring the half-life of glyphosate in human urine samples
Document No	doi.org/10.1016/j.ijheh.2018.09.004 E-ISSN: 1618-131X
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG:** Yes/Reliable with restrictions **Conclusion AGG:** The study is considered to be reliable with restrictions

Full summary of the study according to OECD format

This study aimed to estimate the human half-life of glyphosate from human urine samples collected from amenity horticulture workers using glyphosate based pesticide products.

Materials and methods

Site description and study population - An occupational urinary biomonitoring study for glyphosate was carried out from September 2016 to September 2017. Sample collection took place at the Irish Commissioner for Public Works (OPW) field sites. The tasks completed by the workers sampled were subdivided into three similar exposure groups (SEGs) based on the application method used by the workers to apply glyphosate based pesticide products: manual knapsacks, pressurized applicators and controlled droplet applicators, all of which involved the use of a handheld lance.

Urine collection - Full void urinary spot samples were collected from the participants for 29 work tasks and collected and analysed separately. A minimum of 3 urine samples were collected from each participant: one pre-task sample, one post-task sample and the first morning void sample obtained the day after completing the work task (following first morning void). Participants had an option to provide individual spot urine samples for all urinary voids from the start of the pesticide task to the following first morning void. A pre-labelled sample container was given to every participant for each void and they were asked to write the time and date on the container label. The volume of each urine sample was recorded and the sample was well mixed before taking a 20 mL aliquot to be transferred into a Sterilin™ pot, labelled with a unique identifier number, date and time. Care was taken to avoid any contamination. All samples were stored frozen at -18°C within 24 hours of collection pending analysis.

Urine sample analysis - Glyphosate was extracted from diluted urine samples (200 µl added to 800 µL deionized water) using anion exchange solid phase extraction (SPE) using 10% formic acid in methanol as an eluent. Quantitative analysis was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Chromatographic separation was achieved on a Zorbax XDB-C8, 150×4.6 mm, 5 µm column with 0.1% formic acid and acetonitrile with a gradient elution as the mobile phase. The analytical method was linear over the range 0–20 µg L^{-1} and intra- and inter-assay coefficient of variations (CVs) of 3.54% (n = 10) and 9.96% (n = 40, over 4 runs) were achieved. The analytical limit of quantification (LOQ) was 0.5 µg L^{-1}. Creatinine analysis was performed on all urine samples with a Pentra 400 clinical analyser using the alkaline picrate method.

Estimation of the half-life time of glyphosate - To explore the elimination rate and to estimate the potential human biological half-life of glyphosate, only work tasks with at least two spot urine samples collected after the peak exposure were included for excretion profile analysis. The peak urinary exposure value was defined as the highest urinary glyphosate concentration measured in a spot sample after application per task. The elimination time and the estimation of the half-life of glyphosate was assessed using three different measurement metrics: unadjusted glyphosate concentrations (µg/L), creatinine corrected concentrations (µmol/mol creatinine) and Urinary Excretion Rates (UER). The UER (µg/hr) was calculated by taking the glyphosate concentration of the spot urine sample and multiplying it by the volume of the void and dividing it by the time that elapsed between this urine void and the last urine void.

Statistical analysis - All statistical analyses were performed on Microsoft Excel and Stata Software. Glyphosate concentrations were log transformed as the data showed a log normal distribution. The period of peak sample collection was taken as the start time (t = 0) and the time period from the sample collection time (t = 0) to each proceeding sample was calculated. The slope of the glyphosate urine concentration by the time duration (time passed from the start time) was calculated for each task. Linear interpolation using regression analysis was also performed for each of the included tasks. The mean values, as well as the 95% confidence interval of the half-lives were calculated to estimate the half-life range for each measurement metric.

Results

Urine samples from 7 participants performing 8 work tasks involving glyphosate based products were analysed. Data from 6 males and one female worker is included in this study. One male participated twice on two consecutive days. The age range was from 32 to 60 years, with an arithmetic mean (AM) of 48 years. Workers carried out work tasks that involved the application of glyphosate based pesticide products within one of the SEGs, which lasted between approximately 1 and 6 hours daily. The total sampling time duration of the selected

8 work tasks included in the data analysis, ranged from approximately 19 to 26 hours. In total, 28 individual spot urine samples were analysed for the 8 work tasks included in this study (3 to 4 spot urine samples per sample set). Each sample set was analysed to evaluate the relationships between the measured urinary glyphosate concentrations (µg/L, µmol/mol creatinine or UER) and the duration. The duration started from the peak concentration sample (start time) to each of the subsequent samples. Correlations and linear regression analysis were performed for each sample set. Four sample sets were excluded from the analysis: two creatinine corrected samples sets (µmol/mol creatinine) and two UER calculated sample sets. One creatinine corrected sample set was excluded due to low creatinine levels (< 3 mmol/L) in individual spot urine samples and another because there was no association between concentrations and duration of sampling. This lack of association could relate to a number of factors like gender, diet and hydration.

Each of the sample sets showed a moderate to strong relationship between concentration and duration for all samples (R^2 = 0.42–1.00), with an estimated half-life ranging approximately from 1.5 to 10 hours for unadjusted values or from 4.75 to 20 hours for creatinine corrected values. When the results were restricted to sample sets which showed very strong relationships (R^2 > 0.90), the estimated half-life average (range) was 4.5 (1.5 - 7) hours and 7.5 (4.75 – 9.25) hours for unadjusted and creatinine corrected values, respectively. UER calculated samples showed moderate to strong relationship (R^2 = 0.60–0.95), with an estimated half-life average (range) of 7.25 (3 and 9.50) hours. The average glyphosate half-life including all measuring metrics was approximately 5.5 to 10 hours. The average and range of the half-life on sample sets (numbers 2, 12, 19 and 30) that had all three measuring metrics included was calculated. Sensitivity analysis on the four sample sets, common across all measuring metrics, had an estimated half-life average (range) of approximately 6.5 (4–10), 11.75 (7.25 - 20), and 6.5 (3–7.75) hours for the unadjusted glyphosate concentrations, creatinine corrected concentrations and urinary excretion rate. The limitations of this study are the lack of standardization (pesticide products used, quantity of pesticides applied per task, different application methods and different sampling times). The small sample size prevented the use of more elaborate statistical tests to identify differences due to sex or age. The pharmacokinetic analysis revealed first order kinetics but due to the collection of urine samples over a limited period of time (19-26 hours) multi-phasic kinetics may not have been identified.

Conclusion

The results from this study provide new information on the elimination rate and estimated human biological half-life of glyphosate based on the analysis of urine samples collected during an exposure assessment study. This information can be helpful in the design of sampling strategies, as well as assisting in the interpretation of results for human biomonitoring studies involving glyphosate. The data could also contribute to the development or refinement of Physiologically Based PharmacoKinetic (PBPK) models for glyphosate.

Assessment and conclusion by applicant:

Analytical data for glyphosate obtained from spot urine samples collected during a glyphosate exposure study (Connolly et al., International journal of Hygiene and Environmental Health (2018), Vol. 221, 1012-1022) were used to estimate the human biological half-life of glyphosate. To that end only work tasks with at least two spot urine samples collected after the peak exposure were included for excretion profile analysis. Glyphosate concentrations were log transformed and the slope of the glyphosate urine concentration by the time duration (time passed from the start time) was calculated for each task. When the results were restricted to sample sets which showed very strong relationships ($R^2 > 0.90$), the estimated half-life average (range) was 4.5 (1.5 - 7) hours and 7.5 (4.75 - 9.25) hours for unadjusted and creatinine corrected values, respectively. UER calculated samples showed moderate to strong relationship ($R^2 = 0.60–0.95$), with an estimated half-life average (range) of 7.25 (3 and 9.50) hours. This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions in view of the limitations of the study such as the lack of standardization (pesticide products used, quantity of pesticides applied per task, different application methods and different sampling times). The small sample size prevented the use of more elaborate statistical tests to identify differences due to sex or age. The pharmacokinetic analysis revealed first order kinetics but due to the collection of urine samples over a limited period of time (19-26 hours) multi-phasic kinetics may not have been identified.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions in view of the limitations of the study such as the lack of standardization (pesticide products used, quantity of pesticides applied per task, different application methods and different sampling times). The small sample size prevented the use of more elaborate statistical tests to identify differences due to sex or age. The pharmacokinetic analysis revealed first order kinetics but due to the collection of urine samples over a limited period of time (19-26 hours) multi-phasic kinetics may not have been identified.

Reliability criteria of exposure studies from the applicant

Publication: Connolly *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	Urine monitoring data from a glyphosate exposure study were used to study the urinary excretion kinetics of glyphosate. There are limitations in the study approach.
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	
Exposure to formulations with glyphosate combined with other a.s.	N	
Exposure to various formulations of pesticides	N	
Study		
Study design clearly described	N	Based on the glyphosate exposure study.
Population investigated sufficiently described	N	Based on the glyphosate exposure study.
Exposure circumstances sufficiently described	N	Based on the glyphosate exposure study.
Sampling scheme sufficiently documented	Y	Based on the glyphosate exposure study.
Analytical method described in detail	Y	
Validation of analytical method reported	Y?	Could be more elaborate.
Monitoring results reported	Y	
Statistical analysis	Y	
Pharmacokinetic analysis	Y	To some extent, supposing first order one-compartment pharmacokinetics.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions in view of the limitations of the study such as the lack of standardization (pesticide products used, quantity of pesticides applied per task, different application methods and different sampling times). The small sample size prevented the use of more elaborate statistical tests to identify differences due to sex or age. The pharmacokinetic analysis revealed first order kinetics but due to the collection of urine samples over a limited period of time (19-26 hours) multi-phasic kinetics may not have been identified.		

Assessment and conclusion by RMS:
Agreed with the assessment and conclusion by the applicant. The study is considered reliable with restrictions. The study authors derived an average glyphosate half-life of approximately 5.5 to 10 hours. The present study provides information that may help inform the design of biomonitoring sampling strategies.

B.6.9.8.3. Literature study 3

Data point:	CA 5.9/003
Report author	Connolly, A. *et al.*
Report year	2017
Report title	Exposure assessment using human biomonitoring for glyphosate and fluroxypyr users in amenity horticulture
Document No	doi.org/10.1016/j.ijheh.2017.06.008 ISSN: 1618-131X
Guidelines followed in study	None
Deviations from current test guideline	NA
Previous evaluation	Non-GLP
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Reliable without restrictions **Conclusion AGG:** The study is considered to be reliable without restrictions

Full summary of the study according to OECD format

This study aims to measure occupational exposures to amenity horticulturalists using pesticides containing the active substances, e.g. glyphosate by urinary biomonitoring. A total of 40 work tasks involving glyphosate were surveyed over the period of June – October 2015. Pesticide concentrations were measured in urine samples collected pre and post work tasks using liquid chromatography tandem mass spectrometry. Pesticide urinary concentrations were higher than those reported for environmental exposures and comparable to those reported in some agricultural studies. Log-transformed pesticide concentrations were statistically significantly higher in post-work samples compared to those in pre-work samples. Urinary pesticide concentrations in post-work samples had a geometric mean / geometric standard deviation of 0.66 / 1.11 μg L^{-1}. Linear regression revealed a statistically significant positive association to exist between the time-interval between samples and the log-transformed adjusted (i.e. post-minus pre-task) pesticide urinary concentrations ($\beta = 0.0039$; $p < 0.0001$).

Materials and Methods

Site description and study population - This study was conducted over the period of June – October 2015 in the Republic of Ireland, at field sites managed by the Office of Public Works (OPW). A walk through survey was performed by the researcher at the selected OPW sites including national parks, ornamental gardens and historic monuments, to collect information on the frequency of use of pesticides containing glyphosate and spraying methods used. In this study 3 similar exposure groups were considered for glyphosate applications: glyphosate with a manual knapsack (8 participants using Roundup Biactive XL, 360 g/L; Pistol, 250 g/L; Destrol Amenity, 250 g/L; Glyfos, 360 g/L; Rambo 360, 360 g/L), glyphosate with a controlled droplet applicator (4 participants using Nomix Dual, 120 g/L), and glyphosate with a pressurized handheld lance (5 participants using Roundup Biactive XL, 360 g/L; Pistol, 250 g/L; Glyfo, 360 g/L; Rambo 360, 360 g/L). Amenity horticulturists (age ranging from 33–66 years) who used glyphosate and worked with the OPW at the designated sites, were invited to participate in the study. Participation in the study was voluntary and recruitment was done in coordination with the OPW Health and Safety Unit. Prior to the commencement of the study, the workers were informed of the sampling protocols and methods and completed a self-administered questionnaire to collect information on the participants including their work activities, out-of-work use of pesticides, dietary habits and smoking status.

Biological Monitoring

Urine collection - On the day workers participated in the study, they were asked to provide a pre-work and a post-work sample of up to 50 mL each. The post-work sample was taken within one hour after completion of the task. The samples were stored at -18°C until laboratory analysis. The sampling time in this study was defined as the time interval between the pre- and post-sample collection. Following information was collected for each task:

sampling time, application method, pesticide used, personal protective equipment (PPE) used and climatic conditions. Any changes in PPE, change in weather conditions, breaks taken and problems were also recorded.

Urine sample analysis - Glyphosate standard and glyphosate internal standard (2-$^{13}C^{15}N$-glyphosate) were purchased commercially. 500 µL of urine sample was diluted with 500 µL of deionised water for the analysis of glyphosate. The prepared samples were spiked with 10 µL of internal standard (500 µg/L). Glyphosate was extracted from urine by solid phase extraction (SPE) with subsequent analysis by liquid chromatography tandem mass spectrometry (LC–MS/MS) using a Zorbax SB-C3 150 × 4.6 mm, 5 µm column equipped with a C18 guard column. Mass spectrometry analysis was performed in negative MRM mode. The method was linear over the calibration range 0–20 µg/L. The intra and inter assay coefficients of variation were 3.54% (n = 10) and 9.96% (n = 40, over 4 runs) for glyphosate. The limit of detection (LOD) for glyphosate was set at 0.5 µg/L. Creatinine analysis was performed on all urine samples. Statistical analysis for this study was performed using STATA software. For all exposure measurements below the LOD a single random imputation method was applied to substitute left censored measurements with a value between 0 and the LOD randomly generated from the log normal distribution. Urinary glyphosate concentrations are expressed as geometric means (GM) and geometric standard deviations (GSD) along with arithmetic mean (AM), minimum and maximum levels. Statistical analysis was conducted using biological monitoring data expressed as pesticide urinary concentrations and repeated with the creatinine corrected values. To determine whether there was a significant difference between the log-transformed pre and post task samples, a paired students t-test was conducted. Analysis of Variance (ANOVA) was used to examine differences in urinary pesticide concentrations between the four SEGs, whereas relationships between work characteristics, tasks and urinary pesticide concentrations were examined in classical linear regression. When appropriate, Mann-Whitney tests were also applied to examine differences in continuous variables.

Results

Demographic and working characteristics - The study population consisted of 18 horticulture amenity gardeners (17 men and one woman), who applied pesticides as part of their work. One participant stated that he was a smoker. In 58% of the tasks sampled, a pesticide task had also been completed on the previous day. Of the 40 tasks analysed in this study, 93% of the workers applied glyphosate by spraying with 53% being involved with mixing and loading. The vast majority of the workers wore PPE for each task: 94% wore gloves, 83% respiratory protective equipment (RPE), and 67% Tyvek suits. 89% of the workers reused their PPE and 78% of the workers did not take breaks during the task. The average duration of exposure to glyphosate (from pre-work to post-work sampling time) was 110 minutes for application with a manual knapsack, 235 minutes for application with a large droplet applicator, and 219 minutes for application with a pressurized lance applicator.

Urinary glyphosate concentrations - A total of 80 samples were collected in this study, 40 pre-work and 40 post-work samples. 58% (23 samples) of the pre-work samples and 43% (17 samples) of the post-work samples had glyphosate concentrations below the LOD. For the combined exposure groups the geometric mean was 0.42 µg/L for the pre-work samples and 0.66 µg/L for the post-work samples. The geometric means of the post work glyphosate urinary concentrations of the knapsack and pressurized lance applicators were comparable (0.62 µg/L and 0.57 µg/L, respectively) whereas the geometric mean of the post-work samples of the controlled droplet applicators was higher (1.00 µg/L) although not statistically significantly different from the other exposure groups. The urinary glyphosate concentrations (both simple and corrected for creatinine values) of the pre- and post-work samples were statistically significantly different. When the effects of the time between the collection of the pre- and post-work samples on the urinary concentrations were considered, a positive statistically significant association was observed. Similar results were found with the creatinine corrected values Similar but somewhat less pronounced associations were observed for the controlled droplet applicator and pressurized lance groups, but not for measurements performed during spraying with the manual knapsack. Pesticide urinary concentrations from workers who took breaks during the task were significantly higher by a factor 1.7 on average, compared to those from workers who did not take breaks. Trends were similar when analysis was repeated using the creatinine corrected values. Similarly, median exposure durations were statistically significantly longer for the measurements where workers took breaks compared to those who did not (273 versus 105 minutes), as well as those samples that had pesticide concentrations above the LOD compared to those with concentrations below the LOD (170 versus 98 mins).

Discussion

Studies quantifying occupational exposure to pesticides in amenity horticulture are very sparse. The sensitivity of the analytical method used for glyphosate (LOD of 0.5 µg/L) was comparable to those previously reported. The study results suggest that amenity workers have elevated urinary pesticide levels for glyphosate, above what would be expected from dietary exposures. In 43% of the post-work samples, pesticide concentrations were lower than the LOD. Although very low, the pesticide concentrations in almost all of the post-work samples were higher than those in the pre-work samples. Although no human biomonitoring data are available for the Irish population, the arithmetic mean of the urinary concentrations in the post-work samples (1.35 µg/L) is higher than the mean of 0.21 µg/L reported in an European environmental exposure study. Similarly, both mean and maximum values reported in the current study (1.35 µg/L and 10.66 µg/L, respectively) are also higher than the maximum urinary glyphosate concentrations (0.41 µg/L) reported for German adults with non-occupational exposures to glyphosate. Compared to previous studies among working populations, glyphosate urinary concentrations are comparable to those reported on agricultural exposures and slightly lower but within the range of urinary concentrations reported in studies among farm families in Iowa and farmers in Minnesota and South Carolina. The mean urinary glyphosate concentrations were comparable across all exposure groups although the exposure data from the controlled droplet applicator group appeared to be somewhat more variable than those from the other groups. The observed variation in exposure within this group may reflect inconsistent use of PPE and complacent work practices. Protective coveralls were only worn for 29% of the tasks in this group compared to at least 50% in the other groups.

Previous studies have associated higher pesticide exposures with the mixing and loading of pesticide concentrate. In this study, the majority of the participants performed mixing and loading of the pesticide concentrate as part of the overall task assessed with the exception of the controlled droplet applicators who used a pre-mixed solution. It was therefore not possible to evaluate pesticide exposure during the mixing and loading of the pesticide concentrate. A strong association was found between urinary glyphosate concentrations and exposure duration. The duration of exposure for work involving spraying with the manual knapsack and controlled droplet applicator groups ranged from 5 to 115 min and 33–195 min, respectively. No association between urinary pesticide levels and exposure duration was found for manual knapsack applicators. These study results show that there is a potential for exposure during tasks in horticulture and amenity gardening that typically involve small volumes of pesticides, ranging from 100 mL to 2 L, which warrants further investigation. 89% of the workers reused PPE such as coveralls, gloves, disposable face masks and this may have contributed to the level of exposure. Higher levels of urinary pesticide levels were found among workers who took breaks during the task. In many cases, breaks within the task were required so that the operator could drive or walk to another field site or to take a phone call; usually PPE was not removed during such breaks. Workers who encountered problems during pesticide application such as adjusting the nozzle, leaks or spills, change in climatic conditions or issues with PPE, had higher average urinary concentrations of glyphosate. The sampling strategy adopted in this study (spot sampling pre- and post-work), most likely underestimates the exposure potential. A larger study incorporating 24–72 h urine samples would provide more reliable estimates of exposure and allow comparison with ESFA's designated ADIs and AEOLs.

Conclusions

The results from this study provide evidence of occupational exposure to glyphosate among amenity horticultural workers. The measured levels of urinary concentrations are comparable to those reported for agricultural workers. Urinary concentrations appeared to be dependent on the duration of exposure and the levels measured were higher among workers who took breaks or performed longer tasks, such as the use of controlled droplet applicators. Further research is currently underway to investigate 24 h exposures, evaluate dermal and inadvertent ingestion exposure and their contribution to total body burden of the pesticides.

Assessment and conclusion by applicant:

Biological monitoring has previously been used in studies evaluating occupational exposures to pesticides in both the agricultural and horticultural sectors. The aim of this study was to characterise the occupational exposures in amenity horticultural workers using a biomonitoring method for glyphosate in urine. The geometric mean of the urinary glyphosate concentrations in the post-work samples of all exposure groups combined was found to be 0.66 µg/L. When the relationship between urinary concentrations of glyphosate and systemic dose as established by Acquavella et al. (Acquavella et al. (2004) Environmental Health Perspectives, 112(3), 321-326) is taken into consideration, the daily systemic dose for the workers in this study is estimated to be 0.000021 mg/kg bw/day. The corresponding daily oral external dose is about 0.0001 mg/kg bw/day when an oral

bioavailability of 20% is taken into account. This is 5,000 times lower than the ADI of 0.5 mg/kg bw/day.

This publication is considered relevant for glyphosate risk assessment and reliable without restrictions because it complies with all the reliability criteria of an exposure study.

Reliability criteria for exposure studies from the applicant

Publication: Connolly *et al*., 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.		
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides	Y	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with all the reliability criteria of an exposure study.		

Assessment and conclusion by RMS:
As stated in the study report, the study reports on occupational exposure to glyphosate after spot treatment. In this study, urinary glyphosate concentrations in post-work samples had a geometric mean of 0.66 µg/L. A limitation of the study is that no information on the bodyweight of the participants was included.
In line with the applicant, the RMS considers the study as reliable without restrictions.

B.6.9.8.4. Literature study 4

Data point:	CA 5.9/004
Report author	Connolly, A. *et al.*
Report year	2018a
Report title	Glyphosate in Irish adults – A pilot study in 2017
Document No	doi.org/10.1016/j.envres.2018.04.025 E-ISSN: 1096-0953
Guidelines followed in study	None
Deviations from current test guideline	Not applicable

Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG:** Yes/Reliable without restrictions **Conclusion AGG:** The study is considered to be reliable without restrictions

Full summary of the study according to OECD format

The objective of this study was to conduct an exploratory glyphosate exposure assessment study among Irish adults, who were non-occupational users of glyphosate. A biomonitoring survey involving the collection and analysis of 20 ml spot urine samples from 50 Irish adults was conducted in June 2017. Participants completed a short questionnaire to collect information on demographics, dietary habits and lifestyle. Glyphosate was extracted using solid phase extraction (SPE) and analysed by liquid chromatography tandem mass spectrometry (LC-MC/MS). Of the 50 samples analysed, 10 (20%) contained detectable levels of glyphosate (0.80 – 1.35 μg/L). Exposure concentrations were higher than those reported in comparable studies of European and American adults.

Materials and methods

Using a convenient sampling method, 50 Irish adults (> 18 years) were recruited from 16 counties across the Republic of Ireland to participate in the study over June – August 2017. Participants with specific dietary habits (vegetarian/vegan) and those whose occupation involved the use of pesticides were excluded from the study, to prevent dietary exposure bias, due to a proportionally increased intake of wheat products, fruits and vegetables or from occupational use of glyphosate-based pesticide products. Participants completed a project questionnaire and provided one, 20 mL first morning void urine sample. A questionnaire was designed to collect information on participant demographics, dietary habits and lifestyle.

Glyphosate was extracted using solid phase extraction (SPE) and analysed by liquid chromatography tandem mass spectrometry (LC-MC/MS). The method was linear over the calibration range 0 – 20 μg/L, the limit of detection (LOD) was set at 0.5 μg/L (signal to noise ratio of ≥ 3:1). Creatinine analysis was completed on all samples using an automated alkaline picrate method.

Results

Of the 50 study participants, 60% (n = 30) were female, the mean age was 42 (range 18 − 82), 60% (n = 30) were pet owners and only 10% (n = 5) were smokers. Participants reported that 70% (n = 35) of them consumed few or no portions of organic food. A large proportion of participants, 80% (n = 40), reported consuming 2 portions or less of bread per day.

Home use of pesticides was reported by 40% (n = 20) of participants and 16 of the 20 reported using glyphosate-based products such as Roundup, Gallup, and Weedol, they also reported wearing personal protective equipment when using pesticides (gloves, facemasks or both).

Of the 50 samples analysed, 10 samples (20%) contained detectable levels of glyphosate (0.79 – 1.32 μg/L). Based on results from urinary creatinine analysis, 47 samples were valid (creatinine values < 3.0 or > 30 mmol/L). The three invalid samples had no detectable level of glyphosate. Six of the detectable samples were from females, similar to the gender spread in the full data set. Of the detectable results, 3 out of the 10 participants used glyphosate-based products at home but none of them had used pesticides for at least a month previous (Table 1).

Table 1. Summary of the urinary glyphosate concentrations expressed as µg/L and as µmol/mol creatinine among 50 Irish adults sampled in 2017

Variable		
Samples	**Glyphosate**	
No. of samples analysed *n (%)*	50 (100)	
No. of valid samples *n (%)*	47 (94)	
No. of samples > LOD *n (%)*	10 (20)	
*Sample analysis of 10 samples > 0.5 µg L^{-1} (LOD)	µg L^{-1}	µmol/mol creatinine
Median	0.87	0.41
Maximum	1.35	0.89
Minimum	0.80	0.21

* 20% (10) of the samples analysed had detectable levels.

Conclusion

The proportion of detectable urinary glyphosate concentrations for samples collected from 50 Irish adults is low (20%), which could be due to less localised use of pesticides. A European study that had an LOD of 0.1 µg/L, reported low detection rates in Germany (32%), whereas in the USA, 93% of samples were above 0.5 µg/L (despite an LOD of 0.1 µg/L). The detection rate could possibly have underestimated the true population exposure proportion due to the small sample size and the higher limit of detection of the analytical method used in this study.

Assessment and conclusion by applicant:

This study is newly submitted for purpose of review. A biomonitoring survey involving the collection and analysis of 20 ml spot urine samples from 50 Irish adults on non-occupational setting was conducted. The LC-MC/MS analyses of urinary samples revealed that 10 out of 50 samples analysed (i.e. 20%) contained detectable levels of glyphosate (0.80 – 1.35 µg/L). The low proportion of detectable glyphosate levels could be due to lower localised use of pesticides, having a small sample size or the higher analytical detection limit used in this study (0.5 µg/L).

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good exposure study.

Reliability criteria of exposure studies by the applicant

Publication: Connolly *et al*., 2018.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	?	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	
Exposure to formulations with glyphosate combined with other a.s.	Y	
Exposure to various formulations of pesticides	Y	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	First morning urine void sample.
Analytical method described in detail	Y	To some extent, ref. to other paper.
Validation of analytical method reported	Y	To some extent, ref. to other paper.
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good exposure study.		

Assessment and conclusion by RMS:
The RMS agrees with the assessment and conclusion by the applicant. The study considered reliable without restrictions. The study provides information on non-occupational exposure to glyphosate in the general adult Irish population by urine sampling.

B.6.9.8.5. Literature study 5

Data point:	CA 5.9/005
Report author	Connolly, A. *et al.*
Report year	2019b
Report title	Evaluating glyphosate exposure routes and their contribution to total body burden: a study among amenity horticulturalists
Document No	doi: 10.1093/annweh/wxy104 E-ISSN: 2398-7316
Guidelines followed in study	None

Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG:** Yes/Reliable without restrictions **Conclusion AGG:** The study is considered to be reliable without restrictions

Full summary of the study according to OECD format

The purpose of this study was to evaluate determinants of dermal and inadvertent ingestion exposure and assess their contribution to total body burden among amenity horticultural users using glyphosate-based pesticide products. A dermal and inadvertent ingestion exposure assessment was completed alongside a biomonitoring study among amenity horticultural workers. Linear mixed effect regression models were elaborated to evaluate determinants of exposure and their contribution to total body burden.

A total of 343 wipe and glove samples were collected from 20 workers across 29 work tasks. Geometric mean (GM) glyphosate concentrations of 0.01, 0.04 and 0.05 µg/cm^2 were obtained on wipes from the workers' perioral region and left and right hands, respectively. For disposable and reusable gloves, respectively, GM glyphosate concentrations of 0.43 and 7.99 µg/cm^2 were detected. The combined hand and perioral region glyphosate concentrations explained 40% of the variance in the urinary (µg/L) biomonitoring data. Data show the dermal exposure is the prominent route of exposure in comparison to inadvertent ingestion but inadvertent ingestion may contribute to overall body burden.

Materials and methods

Site description and study population
Exposure assessments were conducted at sites managed by the Irish Commissioner for Public Works (OPW) from September 2016 to September 2017. Workers were grouped into three similar exposure groups (SEGs): manual knapsack, pressurised lance and controlled droplet applicator, based on the applicator used to apply glyphosate-based pesticide products. The manual knapsack applicator SEG (typical capacity of 10 – 15 L), was carried as a knapsack with the pesticide product being applied with a handheld lance. The pressurised lance SEG applied the pesticide product using a handheld lance connected to a motorised knapsack. The controlled droplet applicator SEG, similar to the manual knapsack, but with a capacity 5 L, was purchased with a pre-mixed solution (eliminating the mixing and loading task) and had an adaptable applicator that could increase the droplet size, thus reducing the spray drift.

Study participants were recruited via oral presentation and circulated project information leaflets. Participation was voluntary and all participants gave informed consent. Ethics approval for this project was received from the National University of Ireland, Galway Research Ethics Committee (Ref: 16 July 2019) on the 5th September 2016.

Biomonitoring samples
A biomonitoring study involving the collection of individual full urinary void spot samples was completed and previously been reported (Connolly *et al.*, 2018). A minimum of three urine samples were collected from each participant: a sample before the task began, within 1 h of task completion and the following first morning void. For 59% (n = 17) of tasks, participants gave samples for each void over the exposure assessment period (pre-task to the following first morning void). Urine samples were analysed separately for glyphosate, so the sample with the highest glyphosate concentration could be identified for each task. The urine sample with the highest glyphosate concentration measured during the sampling period was selected and referred to as the peak urinary sample for that participant.

Dermal and inadvertent ingestion sampling strategy
Wipe samples of the hands, perioral region and of potentially contaminated work surfaces (pesticide product container, worker mobile phones and steering wheels of work vehicles) were collected. Wipe sampling was conducted using Ghost Wipes™, pre-packaged polyvinyl alcohol wipes wetted with deionised water by the manufacturer. Dermal and perioral wipe samples were collected from the workers before and after the sampling task. Pre-work task wipe samples were required to evaluate whether detectable data collected post-task was as a

result of the observed pesticide application task. Workers' glove and surface wipe samples were collected after the pesticide application tasks. The researcher wore disposable nitrile gloves for collecting samples and changed these gloves with each sample obtained. An appropriate number of Ghost Wipes™ and glove field blanks were also collected.

Following sampling, wipes were placed in 100 mL plastic pots and appropriately labelled. Samples were extracted and aliquots were frozen to −18°C within 24 h of collection, shipped and chemical analysis completed at the Health and Safety Executive's Laboratory, Buxton, UK.

Dermal sampling
Hand wipes were collected, using two wipes per hand. The front of the hand was wiped with five strokes from the base of the hand to the top of the palm and then five strokes across the palm, starting from the base of the palm of the small finger. The wipe was folded in half and the same sequence repeated on the back of the hand. The wipes were then folded once more and each individual finger was wiped, starting at the small finger to the thumb, going between the fingers and including the finger web areas. Followed by the tips of each finger wiped in a circular motion. The same procedure was completed again with a second wipe and repeated for both hands. Dermal wipe samples were collected from the hands when workers removed their gloves, either during the task (i.e. lunch break) or after the pesticide application task.

Glove contamination samples
PPE use varied from disposable to reusable chemical resistant nitrile gloves as per company policy. After the work task, disposable glove samples were collected for glyphosate analysis, while only some participants provided their reusable gloves for analysis. Worker gloves were collected after the pesticide application tasks or within the task if gloves were changed during the pesticide task. At the end of the pesticide application task, dermal wipe samples were collected of each hand after the gloves were removed.

Inadvertent ingestion sampling
Perioral wipes were collected starting from the upper lip area and wiped in a clockwise motion around the upper lip and philtrum area and down around to the mentolabial fold to the edge of the mouth of the lower lip area. The wipe was folded in half and similarly wiped in an anti-clockwise direction, starting at the lower lip area and finishing at the upper lip area. One wipe was used for the perioral region.

An inadvertent ingestion observational study was also conducted. The frequency of worker contacts per task, (which in the current study included all surfaces contacted by the worker), frequency of worker hand to mouth contacts, contacts with the body and surrounding area (i.e. potentially contaminated surfaces) were recorded. The frequency of contacts was recorded during only the pesticide task. Worker contacts pre- and post-work task or during work breaks were not recorded.

Potentially contaminated work surface sampling
Potentially contaminated work surfaces were wiped using one Ghost Wipe™, according to an object-specific sampling protocol developed within the study. Specifically, mobile phones were first wiped on the front of the phone, from the top to the bottom of the screen in one stroke. The wipe was folded in half and wiped on the back from top to bottom in one stroke. Finally, the wipe was folded once more and the edge of the phone was wiped, starting at the top right hand corner and completing the full edge in a clockwise motion.

Similarly, work vehicle steering wheel wipe samples were wiped from the top of the steering wheel in a clockwise direction, then folded it in half and repeated in an anti-clockwise direction. The wipe was folded once more and the centre of the wheel was wiped in a clockwise direction and the spokes were wiped from the edge to the base of the steering wheel.

Pesticide product containers were wiped from top to bottom for the full width of the container. The bottom of the container was wiped from right to left in one stroke. The wipe was folded and the top of the container was wiped in a clockwise motion. The container handle and surrounding area were wiped from top to bottom afterwards. The wipe was folded once more and the area around the lid was wiped, and then the lid itself, in a clockwise motion.

Chemical analysis
Wipe samples from the hands and perioral region, as well as the disposable glove samples, were placed into a 100 mL plastic pot and extracted by adding 50 mL of deionised water, shaken vigorously for 30 seconds, then

placed on the Denley Spriamax 5 roller mixer for an hour. A 20 mL aliquot was transferred to a labelled Sterilin™ pot for storage and transport before analysis. Glyphosate was extracted from large reusable gloves at the laboratory. One glove was placed into a grip seal bag with 100 mL deionised water. Bags were placed on a gyratory rocker for 1 h with bags being turned over at 30 min. The liquid contents were then transferred to Sterilin™ pots for storage before analysis. The solubility of glyphosate in water (11.6 g/L at 25°C) made it an appropriate extraction solvent.

All samples were prepared and analysed for glyphosate. Glyphosate was extracted from dermal wipe, surface wipe and glove extracted water samples (100 µL diluted with 900 µL deionised water) using strong anion exchange solid phase extraction eluting into 10% formic acid in methanol. The eluent was evaporated under a stream of nitrogen and reconstituted in 200 µL of 0.1% formic acid. Quantitative analysis was performed by liquid chromatography tandem mass spectrometry. Chromatographic separation was achieved on a Zorbax XDB-C8, 150 x 4.6 mm, 5 µm (Agilent, Stockport, UK) column with mobile phases of 0.1% formic acid and acetonitrile with a gradient elution. The method was linear over the range 1–1000 µg/L and the LOQ was 1 µg/L and the LOD was 0.5 µg/L. Where results exceeded the top of the linear range (1000 µg/L) the samples were repeated with dilutions. The method was reproducible with an intra assay CV of 2.9% (n = 12) and an inter assay CV of 4.2% (n = 42, over four runs).

Data processing and analysis
All 23 wipe field blanks had non-detectable glyphosate concentrations. Seventeen glove field blanks were collected from the sites (as some workers used reusable gloves), six having non-detectable glyphosate concentrations, whereas detectable levels were found in the remainder. For each task with detectable glyphosate levels found on the blank glove, each glove sample within that task was field blank corrected. All the samples were corrected for the sample volume and for the surface area wiped. Although samples were not corrected for recovery efficiencies, the mean recovery percentage for plastic containers, disposable chemical resistant nitrile gloves and mobile phones, spike at 20 µg, was 122, 104 and 125%, respectively. Ghost wipes™ have a mean recovery for three samples spiked at 200 µg of 106%.

The average hand surface area measurements were assigned according to published US EPA guidance (US EPA, 2011). The glove adjustments were assigned in the same manner as the surface area for hands. This assumes a 1070 cm^{-2} for both male hands, or 535 cm^{-2} surface per hand and 890 cm^{-2} for both female hands, or 445 cm^{-2} per hand. Average surface area measurements for the perioral region were assigned as 40 cm^{-2}.

Surface area calculations for the steering wheel were assigned as 1100 cm^{-2} surface area. An average mobile phone surface area of 202 cm^{-2} was calculated using the physical phone dimension measurements, based on the phone type sampled. Similarly, for the product containers, an average surface area value of 2300 cm^{-2} was calculated.

Statistical analysis
Before the conducting statistical analysis, all concentration levels below the LOQ were imputed, in SAS v 9.4 (SAS Institute, North Carolina, USA). A single random imputation method based on maximum likelihood estimation was used. The remainder of the statistical analysis was performed using Stata Statistical Software 15 (StataCorp, 2015).

The data were log normally distributed and thus all statistical analysis was performed with log transformed exposure concentrations. Summary and descriptive analysis was performed on the work demographics and glyphosate concentrations levels for the combined dataset and by SEG. The results for the potentially contaminated surfaces are only shown for the combined dataset.

Pearson's correlation coefficients were estimated to evaluate relationships between glyphosate concentrations on the right and left hand, the dominant hand and both hands combined. Similar tests were performed on the glove data.

A linear mixed effect regression model was elaborated based on exposure determinants for inadvertent ingestion previously identified and evaluated in regression analysis against measurements of metals (Gorman Ng *et al.*, 2017). In this model, the hand contamination and the frequency of contacts per task were entered as fixed effects whereas the worker's id was entered as a random effect to account for correlations between repeated measurements from the same worker. This model had some differences to Gorman Ng *et al.*, 2017 model, including that respiratory protective equipment (RPE) was not considered as it was used by all workers

participating in the study and that we used frequency of contacts per task, not just hand to perioral/oral contacts per task. Further models using a forward model-built approach were elaborated to examine the robustness of the derived model as well as to identify determinants for inadvertent ingestion and dermal exposure and their relative contribution to overall body burden. In these models, parameters were entered sequentially based on their level of significance and kept within the model if they had a statistical significance of ($P < 0.1$).

Results

Demographic and working characteristics
Twenty amenity horticulturists who applied glyphosate-based pesticide products as part of their daily duties participated in the study (18 males and 2 females) were grouped into 3 SEGs. The pesticide task duration ranged from approximately a half hour to 6 hours. Work tasks involving the manual knapsack, controlled droplet applicator and the pressurised applicator were, on average, ~3, 3½ and 6 h, respectively.
Good worker compliance with PPE use was observed, with workers using PPE for most of the work tasks sampled; gloves, Tyvek suits and RPE were used for 29 (100%), 26 (90%) and 28 (97%) of the observed tasks, respectively.

Levels of glyphosate concentrations on wipes, gloves and contaminated surfaces
A total of 343 wipe and glove samples across 29 work tasks were collected and analysed for glyphosate. A minimum of seven sets of wipe samples were collected for each task sampled. A sample set consists of a blank wipe, perioral sample and each hand sample (two wipes per hand), before and after each work task.

Glyphosate concentration data for perioral and hand wipes (µg/cm), collected pre and post the work tasks are presented in Table 1 for overall samples and per SEG. Table 2 details the glyphosate concentration data for the disposable and reusable gloves samples. Seventeen pairs of disposable gloves and seven pairs of reusable gloves were analysed in this study. For three of the work tasks analysed, workers wore disposable gloves over a pair of reusable gloves and gave both sets of gloves for analysis. For eight of the tasks, the workers refused to give their gloves. Glyphosate was detected in all the pre- and post-work task hand wipe samples, as well as on the post-work task glove samples. Only 11 (38%) of the pre-work task and 6 (21%) post-work task perioral wipes had non-detectable glyphosate concentrations. For a third of work tasks sampled ($n = 10$), workers had started the pesticide task prior to the collection of pre-work task samples. Detectable glyphosate concentrations were also found on 11 (65%) of the blank gloves samples, collected from PPE field stores. Field observations suggest that cross contamination may occur when storing new gloves in close proximity of the pesticide chemical storage area or when handling unused gloves with contaminated hands.

In the current study, arithmetic mean (range) glyphosate concentrations of 2708 (3.0–21 845) µg wipe^{-1} and 2797 (5.0–27 354) µg wipe^{-1}, (right and left hand, respectively) were found on worker hand wipe samples collected after the pesticide application task. Glyphosate concentration levels of 41 (< LOQ–321) µg per wipe were detected on wipes collected from the perioral region. Values reported for hand wipes in this study were higher than those found for agricultural pesticide users 647 (83–2081) µg (Christopher, 2008). However, the perioral glyphosate concentrations were within a range of that reported in Christopher (2008), with an arithmetic mean and range of 39.5 (2.6 – 91) µg. Christopher (2008) also detected glyphosate in worker saliva samples with an arithmetic mean and range of 140 (56–440) ng, which could suggest comparable inadvertent ingestion levels for the current study workers.

Strong positive associations were found between left and right hands, and between the dominant hand, the individual hands and the combination of both hands. Between SEGs, similar glyphosate concentrations were detected on perioral wipes, with the highest geometric mean (GM) and maximum value found in the pressurised lance SEG. Within SEGs, similar glyphosate wipe concentrations were found on both the right and left hands in the manual knapsack group and the pressurised lance applicator. Glyphosate concentrations on reusable gloves were two orders of magnitudes higher than those on disposable gloves.

On some occasions, additional dermal and gloves samples were collected for workers who took a break during the pesticide application task. For three of the work tasks, dermal wipe samples were collected from three participants before taking a break and the glyphosate concentrations for the perioral and both hands (combined) ranged from 0.01 to 0.15 and 0.002 to 0.41 µg/cm, respectively. Similarly, three participants provided their disposable gloves during the break for five of the work tasks, which included two of these participants giving samples on two consecutive days and found glyphosate concentrations that ranged from 1.27 to 20.89 µg/cm. As concentrations were similar to the post-work task samples and due to the limited sample numbers, this data were

not included in further analysis. The additional samples collected could only have had a negligible effect on the post-work task samples due to similar detection levels. The assumption would be that workers would wash hands and dispose of contaminated work gloves before their break, removing the contaminant, thus no accumulation of glyphosate concentration occurs on the post-work task samples.

Wipe samples data from pesticide product containers (n = 21), work vehicle steering wheels (n = 10) and participant's personal mobile phones (n = 18) are presented in Table 3. Of all the potentially contaminated work surfaces sampled in this study (n = 49), the highest glyphosate concentrations were detected on the pesticide product containers. A pesticide product container is considered to be handled up to 50 times by the worker before disposal.

Glyphosate was also detectable on all wipes from work vehicle steering wheels (n = 21), with a mean and range of 1928 (478–5984) µg wipe^{-1} (unadjusted values). These included small tractors and vehicles (e.g. vans, cars) used to transport equipment to and around field sites. Participating workers drove the work vehicle, on some occasions to travel to multiple sites within a day, and performed the required pesticide application tasks. Only two (11%) of the mobile phone samples had non-detectable glyphosate concentrations. Most mobile phones were personal use phones.

Table 6.9.8.5-1. Glyphosate wipe concentration data (µg/cm) for pre- and post-work task perioral and hand (left and right) measurements. Results are presented as for the overall sample and per similar exposure group concerned

Variable	*k*	*N*	<LOQ *N* (%)	GM	GSD	Min	Max	<LOQ *N* (%)	GM	GSD	Min	Max
Combined SEGs				Pre-work task samples					Post-work task samples			
Perioral	20	29	11 (38%)	2.1×10^{-03}	12.93	8.2×10^{-06}	0.15	6 (21%)	0.01	9.05	1.7×10^{-04}	0.40
Hand left	20	29	0	6.1×10^{-03}	8.77	9.4×10^{-05}	0.31	0	0.04	9.21	4.7×10^{-04}	2.56
Hand right	20	29	0	6.5×10^{-03}	9.09	9.4×10^{-05}	0.22	0	0.05	8.73	2.8×10^{-04}	2.04
Manual knapsack				Pre-work task samples					Post-work task samples			
Perioral	10	12	6 (50 %)	1.1×10^{-03}	21.30	8.2×10^{-06}	0.15	4 (33%)	0.01	11.30	1.7×10^{-04}	0.23
Hand left	10	12	0	2.2×10^{-03}	8.47	9.4×10^{-05}	0.07	0	0.01	4.64	4.7×10^{-04}	0.08
Hand right	10	12	0	2.7×10^{-03}	11.00	9.4×10^{-05}	0.14	0	0.01	5.26	2.8×10^{-04}	0.09
Pressurised lance				Pre-work task samples					Post-work task samples			
Perioral	6	10	2 (20 %)	3.9×10^{-03}	8.56	5.8×10^{-05}	0.05	0	0.04	4.68	2.5×10^{-03}	0.40
Hand left	6	10	0	2.2×10^{-02}	10.01	3.7×10^{-04}	0.31	0	0.19	9.17	2.6×10^{-03}	2.56
Hand right	6	10	0	1.8×10^{-02}	10.62	4.7×10^{-04}	0.22	0	0.21	7.41	3.7×10^{-03}	2.04
Controlled droplet				Pre-work task samples					Post-work task samples			
Perioral	5	7	3 (43 %)	2.6×10^{-03}	9.44	1.1×10^{-04}	0.03	2 (29%)	0.01	8.34	6.0×10^{-04}	0.10
Hand left	5	7	0	5.9×10^{-03}	2.39	2.4×10^{-03}	0.03	0	0.04	5.89	3.5×10^{-03}	0.50
Hand right	5	7	0	6.8×10^{-03}	2.09	3.0×10^{-03}	0.02	0	0.06	5.73	7.9×10^{-03}	0.85

k is the number of participants in the group. *N* is the number of samples (one sample per task) in the group. Number of samples below the limit of quantification (<LOQ) (1 µg l^{-1}) by number (*N*) and percentage (%). GM is the geometric mean, GSD the geometric standard deviation and the range (min–max).

Table 6.9.8.5-2. Glyphosate wipe concentration data (µg/cm) for the post-work task glove samples. Results are presented for the overall sample and per similar exposure group concerned

Variable	*k*	*N*	GM	GSD	Min	Max	*k*	*N*	GM	GSD	Min	Max
Combined SEGs			Post-work task disposable gloves						Post-work task reusable gloves			
Glove left	12	17	0.18	6.14	3.9×10^{-03}	2.78	6	6	4.49	4.68	0.81	58.87
Glove right	12	17	0.20	6.96	2.4×10^{-03}	10.62	6	6	4.52	4.43	0.73	66.27
Glove both	12	17	0.43	5.67	0.02	13.41	6	7	7.99	4.14	1.53	125.1
Manual knapsack			Post-work task disposable gloves						Post-work task reusable gloves			
Glove left	7	8	0.06	5.39	3.9×10^{-03}	0.63	1	1	—	—	6.11	6.11
Glove right	7	8	0.07	6.56	2.4×10^{-03}	0.49	1	1	—	—	6.43	6.43
Glove both	7	8	0.15	4.46	0.02	1.12	1	1	—	—	12.54	12.54
Pressurised lance			Post-work task disposable gloves						Post-work task reusable gloves			
Glove left	3	4	0.39	2.42	0.19	1.23	2	2	—	—	6.64	58.87
Glove right	3	4	0.36	1.44	0.26	0.58	2	2	—	—	6.11	66.27
Glove both	3	4	0.80	1.94	0.46	1.76	2	2	—	—	12.74	125.1
Controlled droplet			Post-work task disposable gloves						Post-work task reusable gloves			
Glove left	3	5	0.55	5.83	0.04	2.78	2	3	1.51	1.75	0.81	2.39
Glove right	3	5	0.75	7.29	0.05	10.62	2	3	1.49	1.86	0.73	2.17
Glove both	3	5	1.35	6.76	0.09	13.41	3	4	3.19	1.64	1.53	4.56

k is the number of participants in the group. *N* is the number of samples (one sample per task) in the group. There was no samples in this table that were below the limit of quantification (<LOQ) (1 μg l^{-1}). GM is the geometric mean, GSD the geometric standard deviation and the range (min–max).

Table 6.9.8.5-3. Glyphosate concentration data (μg/cm) for wipe samples collected from work surfaces. Sampling from those was performed post-work task completion and results are presented as geometric mean (GM), geometric standard deviation (GSD) and range (min–max) for the overall sample

Variables	*k*	*N*	<LOQ*N* (%)	GM	GSD	Min	Max
Combined SEGs							
Product container	15	21	0	2.06	7.48	0.01	27.7
Steering wheel	7	10	0	0.06	2.44	0.02	0.27
Mobile	15	18	2 (11%)	0.004	6.50	1.0×10^{-4}	0.12

k is the number of participants in the group. *N* is the number of samples (one sample per task) in the group. Samples that are below the limit of quantification (<LOQ) (1 μg l^{-1}) by number (*N*) and percentage (%). GM is the geometric mean, GSD the geometric standard deviation and the range (min–max).

Differences in exposure levels across SEGs, sampling and working parameters

Disposable worker gloves had the highest glyphosate concentrations, followed by worker hands (post-work task). Glyphosate concentrations were lowest on perioral wipes (Fig. 1a). The highest glyphosate concentrations were detected on wipe samples collected from the pesticide product containers, followed by much lower levels on the work vehicle steering wheel. The lowest concentrations were detected on the worker mobile phones (Fig. 1b). Three of the participants in this study had their mobile phone wiped on two separate occasions, and on both occasions, glyphosate concentrations were detected.

A strong positive relationship was found between urinary glyphosate concentrations (μg/L) and glyphosate wipe concentrations (μg/cm) on the worker perioral wipe samples (Fig. 2a). Similarly, perioral region and worker hands glyphosate concentrations (μg/cm) correlated positively and strongly (Fig. 2b). Some of the participants started the work task before monitoring began. Although this has no influence on the dermal exposure assessments measurements as samples were taken before and after the work task but it could potentially impact on the urinary concentrations which may not accurately reflect the full day's exposure. The precision of peak urinary concentration estimates used within our analysis may be dependent on the number of samples available for participants. However, no statistically significant differences were found in peak urinary concentrations between participants.

Figure 6.9.8.5-1. Boxplot showing the post-work task glyphosate concentrations for (a) disposable gloves, the workers hands (under the glove) and the perioral region and (b) for potentially contaminated work surfaces, pesticide product containers, the steering wheel of work vehicles and participants mobile phone (µg/cm). The box is the 25th to the 75th percentile, the line within the box is the median and the whiskers the lower and the upper adjacent values. Single points indicate outliers. *n is the number of samples. Reusable gloves were not included in the boxplot (a) due to the low number of samples. Mean measured concentrations were statistically different between all types of samples (*t*-tests; P < 0.001).

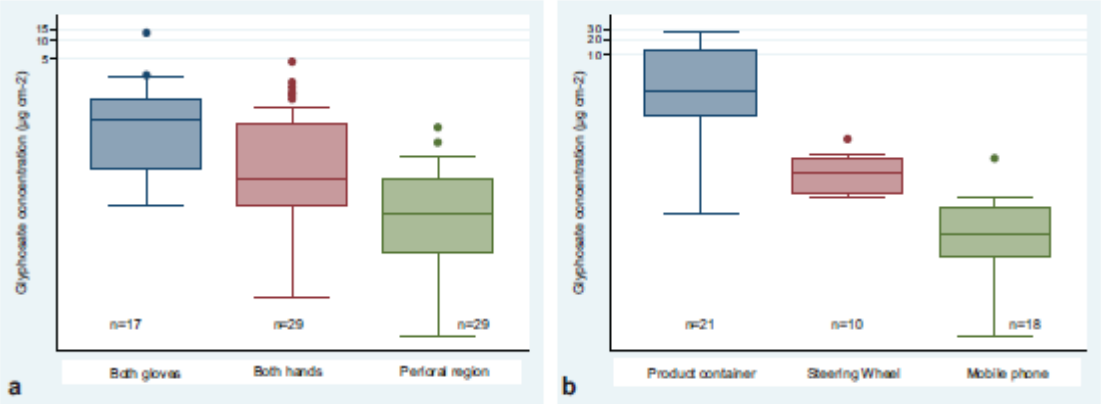
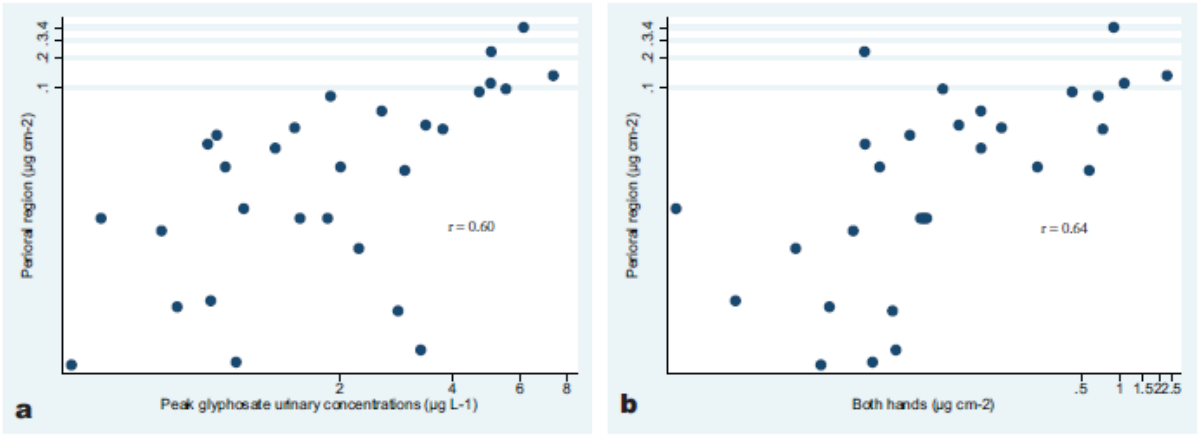


Figure 6.9.8.5-2. Scatter graph showing moderate relationships between glyphosate perioral glyphosate wipe contamination levels (µg/cm) and (a) peak urinary glyphosate concentrations (µg/L) and (b) both hands glyphosate surface loading contamination levels after a pesticide application task (µg/cm).



Elaborated linear mixed effect regression models are presented in Table 4. The model evaluating previously documented determinants of inadvertent ingestion exposure Gorman Ng *et al.* (2017), explained 45% of the variability for the glyphosate concentrations found on the perioral region. In this model (Model 1), an increase in the frequency of contacts per task and post-task hand contamination was significantly associated with an increased in perioral glyphosate concentrations.

Forward building of the same model on the basis of improvement of the fit parameters resulted with the same parameters being included alongside sampling time. The effects for hand contamination and frequency of contacts per task were comparable to those of the Model 1, whereas perioral concentrations also increased with increasing sampling time ($\beta = 0.01$; $P < 0.08$). This model explained ~50% of the total variability in perioral glyphosate concentrations.

The forward built model examining determinants of participants' hand contamination (Model 2) comprised of the task sampling time, the age of the participant and the SEG and explained 62% of the total variability of glyphosate concentrations measured on the hands.

All the workers who participated in this study wore gloves when applying pesticides. Gloves were used throughout the task however it was observed that on a number of occasions workers would remove their gloves for various reasons (e.g. checking mobile phones, to drive work vehicles, when going on a break, etc.). All had detectable glyphosate concentrations on their hands. Field observations suggest that poor glove doffing procedures or removing gloves during the work task (e.g. answering the phone, adjusting facemasks) may be responsible for hand contamination.

Table 6.9.8.5-4. Mixed effect models with participants' identification number included in the models as a random effect. Model 1 results are describing the effects of hand contamination and the frequency of contacts per task on glyphosate concentrations measured on the perioral region. Model 2 results describe the effects of sampling time, the age of the workers and work task characteristic on glyphosate surface loading concentrations measured on the hands. Measurements are given as on the log-transformed glyphosate concentrations per surface area (µg cm−2) and were taken from 20 workers over 29 pesticide application tasks.

Covariate	Model 1			Model 2		
	β	SE	*P*	β	SE	*P*
Intercept	–3.91	0.70	0.00	–10.05	1.67	0.00
Glyphosate conc. of both hands, post-work task (µg cm^{-2})	0.56	0.16	0.00			
Sampling time of the work task (mins)				0.01	0.003	0.01
Total frequency of contacts per task	0.02	0.01	0.02			
Age of the participant (years)				0.12	0.04	0.00
Similar exposure groups						
Pressurised lance				1.41	0.67	0.04
Controlled droplet applicator				0.69	0.67	0.30
Manual knapsack				Ref	—	—
Between variance (naive model)		1.18 (2.32)			0.00 (3.94)	
Within variance (naive model)		1.55 (2.62)			1.85 (0.97)	
Total variance explained		45%			62%	

β, regression coefficient for log-transformed exposure data; SE, standard error. The naive model is the results from the model without the inclusion of any fixed effects.

To identify the relative contribution of the routes of exposure to the total uptake of glyphosate, a separate model was forward built using the log-transformed results of the biomonitoring exposure measurements as the dependent covariate. The final elaborated model comprised of only the combined hand and perioral region glyphosate concentration (Table 5). Overall, the model explained 40% of the total variance in urinary concentrations. The hands and perioral data were not entered as separate covariates in the model, as they were highly correlated ($r = 0.64$; $P < 0.001$). Hand and perioral glyphosate concentrations are important determinants of total glyphosate body burden (glyphosate urinary concentrations), explaining 40% of variance in the urine data. Discriminating the individual contribution for each route to the total body burden was not possible. However, hand glyphosate concentrations alone explained approximately one third of the variance in the glyphosate urine concentrations, which would indicate that dermal exposure was the predominant route but that inadvertent ingestion may contribute to overall body burden since the presence of glyphosate contamination in the perioral region may result in ingestion and/or dermal absorption.

Table 6.9.8.5-5. Mixed effect model describing the effects of glyphosate concentration of the combined hands and the perioral region on the log-transformed glyphosate concentrations (µg/L) measured of 20 workers over 29 pesticide application tasks. Mixed models build with participants' identification number as a random effect.

Covariate	β	SE	*P*
Intercept	1.20	0.19	0.00
Ln concentrations in hand and perioral region surfaces	0.26	0.06	0.00
Between variance (naive model)		0.15 (0.36)	
Within variance (naive model)		0.22 (0.27)	
Total variance explained		40%	

β, regression coefficient for log-transformed exposure data; SE, standard error. The naive model is the results from the model without the inclusion of any fixed effects.

Conclusion

The analysis of a total of 343 wipe and glove samples were collected from 20 workers across 29 work tasks revealed the GM glyphosate concentrations of 0.01, 0.04 and 0.05 μg/cm^2 on wipes from the workers' perioral region and left and right hands, respectively. For disposable and reusable gloves, respectively, GM glyphosate concentrations of 0.43 and 7.99 μg/cm^2 were detected. The combined hand and perioral region glyphosate concentrations explained 40% of the variance in the urinary (μg/L) biomonitoring data. The results of the study show dermal to be a prominent route of exposure but support inadvertent ingestion potential contribution to the total body burden among this worker group. The study also identified a potential for the spread of contamination among non-pesticide users in the workforce and para-occupational exposures. Study results also showed that PPE practice is an important determinant of both inadvertent ingestion and dermal exposure. An implementation of PPE management and work practices policies for pesticide use could potentially reduce both occupational exposures and para-occupational exposures.

Assessment and conclusion by applicant:

This study is newly submitted for purpose of review. The total uptake of glyphosate was assessed in parallel with dermal and inadvertent exposure routes, using urine, wipes and glove samples collected from 20 workers across 29 work tasks. The average hand surface area measurements were assigned according to published US EPA guidance. Geometric mean (GM) glyphosate concentrations of 0.01, 0.04 and 0.05 μg/cm^2 were obtained on wipes from the workers' perioral region and left and right hands, respectively. For disposable and reusable gloves, respectively, GM glyphosate concentrations of 0.43 and 7.99 μg/cm^2 were detected. The combined hand and perioral region glyphosate concentrations explained 40% of the variance in the urinary (μg/L) biomonitoring data. Data show the dermal exposure is the prominent route of exposure in comparison to inadvertent ingestion, but inadvertent ingestion may contribute to overall body burden. The study also identified potential exposure to non-pesticide users in the workplace and para-occupational exposures.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good exposure study.

Reliability criteria of exposure studies from the applicant

Publication: Connolly *et al.*, 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	

Reliability criteria of exposure studies from the applicant

Publication: Connolly *et al.*, 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Exposure to formulations with glyphosate combined with other a.s.	Y	
Exposure to various formulations of pesticides	Y	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good exposure study.		

Assessment and conclusion by RMS:

The RMS agrees with the assessment and conclusion by the RMS. The exposure study is considered reliable without restrictions.

B.6.9.8.6. Literature study 6

Data point:	CA 5.9/006
Report author	Conrad, U. *et al.*
Report year	2017
Report title	Glyphosate in German adults – Time trend (2001 to 2015) of human exposure to a widely used herbicide
Document No	doi.org/10.1016/j.ijheh.2016.09.016100898843 E-ISSN: 1618-131X
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG:** Yes/Reliable without restrictions **Conclusion AGG:** The study is considered to be reliable without restrictions

Full summary of the study according to OECD format

The purpose of this study was to elucidate the internal exposure of the general German population to glyphosate and aminomethylphosphonic acid (AMPA) and its change over time.

The broadband herbicide glyphosate (N-[phosphonomethyl]-glycine) and its main metabolite AMPA were analysed by GC-MS-MS in 24 h-urine samples cryo-archived by the German Environmental Specimen Bank (ESB). Samples collected in 2001, 2003, 2005, 2007, 2009, 2011, 2012, 2013, 2014, and 2015 were chosen for this retrospective analysis. All urine samples had been provided by 20 to 29 years old individuals living in Greifswald, a city in north-eastern Germany.

Out of the 399 analysed urine samples, 127 (= 31.8%) contained glyphosate concentrations at or above the limit of quantification (LOQ) of 0.1 µg/L. For AMPA this was the case for 160 (= 40.1%) samples. The fraction of glyphosate levels at or above LOQ peaked in 2012 (57.5%) and 2013 (56.4%) after having discontinuously increased from 10.0% in 2001. Quantification rates were lower again in 2014 and 2015 with 32.5% and 40.0%, respectively. The overall trend for quantifiable AMPA levels was similar. Glyphosate and AMPA concentrations in urine were statistically significantly correlated (spearman rank correlation coefficient = 0.506, $p \leq 0.001$). Urinary glyphosate and AMPA levels tended to be higher in males. The possible reduction in exposure since 2013 may be due to changes in glyphosate application in agricultural practice.

Materials and Methods

Sampling and study group; This retrospective monitoring study was based on 24 h-urine specimen collected in the annual sampling of the German ESB. To reduce the risk of contamination, all containers needed for sampling and aliquoting were carefully cleaned before use according to standard operating procedures. All samples have been provided by young adults (predominantly students) aged 20 to 29 years. To follow the time trend of human exposure to glyphosate and AMPA, cryo-preserved urine samples collected in 2001, 2003, 2005, 2007, 2009, 2011, 2012, 2013, 2014, and 2015 were analysed. All urine samples were collected from individuals living in Greifswald, a city in north-eastern Germany. Annual ESB sampling in Greifswald was regularly carried out in the period of March and April. From each of the ten study years, 24 h-urine samples donated by 20 male and 20 female participants were randomly selected for analyses. The only inclusion criterion for this main study sample was that no specifically restricted diet – mainly vegetarian or vegan – had been reported by the sample provider in the self-administered ESB questionnaire. In 2001 the questionnaire item on dietary restrictions had not yet been implemented. Therefore, some samples from 2001 may have been provided by vegetarians or vegans. The fraction of vegetarian or vegan ESB participants, however, remained roughly between 2 and 14% from 2002 to 2014 followed by fractions up to 18% in 2015. Therefore, it can be assumed that, if any, only very few participants with restricted diets might have erroneously been included in the 2001 sample. One 2013 measurement had to be excluded from the main study sample, as the participant was later identified not to fulfil the inclusion criterion.

Hence, the main sample of this study consisted of 399 participants living in the ESB sampling location Greifswald with virtually equal sample sizes and sex ratios in each study year (cf. Table 1).

Table 6.9.8.6-1. Description of sample composition (ESB participants from Greifswald analysed for glyphosate and AMPA concentrations in 24 h-urine (no self-reported specific dietary restrictions)

Year	Sample size (male/female)	Age [years] AM (range)	24 h-urine volume [mL] AM (range)	Creatinine in urine [g/L] AM (range)	BMI [kg/m^2] AM (range)
2001	40 (20/20)	23.2 (20–28)	1757 (490–3000)	0.98 (0.38–3.73)	22.3 (17.8–27.4)
2003	40 (20/20)	24.6 (20–28)	1793 (770–2850)	1.06 (0.42–2.11)	23.4 (17.3–31.2)
2005	40 (20/20)	23.1 (20–29)	1910 (895–2841)	0.90 (0.29–2.38)	22.6 (18.1–33.2)
2007	40 (20/20)	23.8 (20–28)	1937 (771–3047)	0.96 (0.36–2.92)	23.3 (18.0–34.6)
2009	40 (20/20)	24.3 (20–29)	1959 (701–3438)	0.85 (0.37–2.39)	22.7 (17.8–34.6)
2011	40 (20/20)	24.3 (20–29)	1893 (783–3045)	0.87 (0.26–2.17)	23.6 (18.0–39.1)
2012	40 (20/20)	24.4 (20–29)	1802 (768–3076)	0.97 (0.32–2.27)	22.8 (17.5–29.8)
2013	39 (20/19)	24.7 (20–29)	1973 (924–3081)	0.75 (0.20–1.60)	23.9 (19.3–40.9)
2014	40 (20/20)	24.1 (20–28)	1958 (760–3069)	0.82 (0.28–2.07)	23.2 (17.9–44.8)
2015	40 (20/20)	24.3 (20–28)	1759 (588–2956)	0.93 (0.18–2.02)	23.0 (17.9–36.8)
Total	399	24.1 (20–29)	1874 (490–3438)	0.91 (0.18–3.73)	23.1 (17.3–44.8)
Male	200	24.4 (20–29)	1881 (490–3076)	1.09 (0.20–3.73)	24.1 (17.4–36.8)
Female	199	23.8 (20–29)	1866 (600–3438)	0.73 (0.18–2.02)	22.0 (17.3–44.8)

Notes: AM = arithmetic mean, BMI = body mass index.

Male ESB participants tended to have higher BMI values and urinary creatinine levels than females. To investigate possible regional/seasonal differences of glyphosate and AMPA levels 40 urine samples collected in

January 2005 and 2015 at the ESB sampling location Muenster (a city in north-western Germany) have additionally been analysed. Moreover, 20 urine samples from vegetarian or vegan participants have been analysed as well, in order to investigate differences due to diet. These samples were collected in Greifswald in the years 2007 (10 females) and 2015 (5 males and 5 females) and represent all available samples from vegetarian or vegan participants. A description of the two additionally analysed comparative ESB sub-populations is provided in Table 2. Participants in Muenster tended to have slightly lower BMI values. The other sub-population of self-reported vegetarians/vegans also exhibited lower average BMI values as well as higher 24 h-urine sample volumes and lower urinary creatinine concentrations.

Table 6.9.8.6-2. Description of two sub-populations from Muenster (no self-reported specific dietary restrictions) and Greifswald (self-reported vegetarians/vegans) analysed for comparison with the main study sample

Year	Sample size (male/female)	Age [years] AM (range)	24 h-urine volume [mL] AM (range)	Creatinine in urine [g/L] AM (range)	BMI [kg/m^2] AM (range)
ESB sampling location Muenster (no self-reported specific dietary restrictions)					
2005	40 (20/20)	23.6 (20–28)	1790 (691–2962)	1.03 (0.19–2.41)	22.2 (17.4–29.3)
2015	40 (20/20)	23.6 (20–28)	1991 (271–4601)	0.75 (0.35–1.73)	22.1 (18.3–28.6)
Total	80 (40/40)	23.6 (20–28)	1891 (271–4601)	0.89 (0.19–2.41)	22.1 (17.4–29.3)
Male	40	24.0 (20–28)	1934 (797–2952)	0.98 (0.19–2.41)	23.1 (19.6–28.6)
Female	40	23.3 (20–28)	1847 (271–4601)	0.80 (0.35–1.65)	21.1 (17.4–29.3)
Self-reported vegetarians/vegans (ESB sampling location Greifswald)					
2007	10 (0/10)	24.5 (23–28)	2293 (457–3011)	0.67 (0.21–2.51)	23.0 (19.9–28.1)
2015	10 (5/5)	24.3 (20–28)	1831 (773–2993)	0.72 (0.24–1.40)	22.1 (17.7–25.1)
Total	20 (5/15)	24.4 (20–28)	2062 (457–3011)	0.69 (0.21–2.51)	22.5 (17.7–28.1)
Male	5	25.4 (24–27)	1915 (1135–2813)	0.80 (0.31–1.36)	22.6 (21.3–23.9)
Female	15	24.1 (20–28)	2111 (457–3011)	0.66 (0.21–2.51)	22.5 (17.7–28.1)

Notes: AM = arithmetic mean, BMI = body mass index.

Analytical procedure; The chemical analysis was based on the method by Alferness and Iwata (1994) initially developed for trace analysis of Glyphosate and AMPA in food which uses gas chromatography (GC) coupled with a single quadrupole mass selective detector (MSD). The newly developed method applied in the present study used GC with tandem mass spectrometry (GC-MS-MS) to reach a low limit of quantification (LOQ) in human urine along with high selectivity. Isotope labelled internal standards have been used for further increasing the method's performance.

Standards and reagents; All chemicals were of analytical grade unless stated otherwise. Reference compounds (glyphosate and AMPA) and internal standards (1,2-13C2-15N-glyphosate and 13C-15N-AMPA) were obtained from Dr. Ehrenstorfer (Augsburg, Germany) as solutions in water (10 g/mL each). 2,2,2-trifluoroethanol (99%), trifluoroacetic anhydride (99%) and acetonitrile were purchased from Sigma-Aldrich (Seelze, Germany). Methanol was obtained from Merck (Darmstadt, Germany). Water was purified by an ultra-water purification system from ELGA (Ransbach-Baumbach, Germany).

Sample preparation; 50 L of urine sample and 25 L of the internal standard (IS) solution (containing 4 ng/mL of each IS) were transferred to 10 mL screw-capped glass tubes containing 1 mL of acetonitrile. After evaporation to dryness in a vacuum centrifuge, 0.5 mL of 2,2,2-trifluoroethanol and 1 mL of freezing cold (-40 °C) trifluoroacetic anhydride were added cautiously to the residue. The derivatization of the analytes was started by heating the closed tubes to 85 ◦C for 1 h in a heating block. After cooling down to room temperature the mixture was cautiously evaporated to dryness. The oily residue was then dissolved in 100 L of methanol and transferred into a microvial. This final solution was used for GC-MS-MS analysis. Mixed glyphosate and AMPA calibration solutions were prepared by serial dilution of a stock solution (each 5 ng/mL) in solutions of 50 L water in 1 mL acetonitrile containing 25 L of the IS-solution. These solutions were processed in the same way as described for human urine samples and represent sample concentrations from 0.1 to 10 µg/L.

GC-MS-MS analysis; The derivatised analytes were separated by gas chromatography using a GC system7890 equipped with a split/split less injector (Agilent) and a MPS2 autosampler (Gerstel). The GC column was a HP INNOWAX, 30 m length, 0.25 mm internal diameter and 0.25 m film thickness (Agilent). The mass spectrometric parameter and ion transitions used are summarised in Table 3. While the primary transitions are well suitable for quantification of glyphosate and AMPA at low environmental internal exposure levels, the secondary transitions of glyphosate and AMPA only worked well at urine concentrations beyond approx. 20 µg/L to confirm the identity of analytes. As the method was clearly focused on reaching the lowest quantification

limits in human urine, the secondary transitions were not considered. The high specificity of the primary ion transition was evaluated during the validation of the analytical method.

Table 6.9.8.6-3. Mass spectrometric parameter and ion transitions used in glyphosate and AMPA analyses

Mass spectrometric parameters	
Instrument	Agilent 7000
Ion source temperature	150 °C
Ionization type	Negative chemical ionization (NCI)
Chemical ionization gas	Methane 4.5
Collision gas	Argon 5.0
Electron multiplier	Relative +200 V

Mass transfers of analytes and internal standards

Transition	Precursor ion [m/z]	Product ion [m/z]	Collision energy [V]	Use
Glyphosate 1st transition	370	245	10	Analyte quantifier
Glyphosate 2nd transition	351	268	5	Of limited suitability
AMPA 1st transition	351	268	5	Analyte quantifier
AMPA 2nd transition	271	188	5	Of limited suitability
1,2-$^{13}C_2$-^{15}N-glyphosate	371	246	10	Internal standard
^{13}C-^{15}N-AMPA	353	270	5	Internal standard

Notes: The secondary transitions of glyphosate and AMPA are listed for sake of completeness only. As they provide reliable confirming information only at concentrations beyond 20 µg/L, they have not been used in this study.

Validation and quality assurance measures of analytical method; For evaluation of the method performance the requirement of SANCO guideline 825 (European Commission, 2010) were considered which are mandatory for analytical methods in the context of pesticide registration and monitoring. Specificity, linearity, working range, accuracy, precision and LOQ were investigated for method evaluation. It can be concluded that the primary transition was very selective for a reliable quantification of glyphosate and AMPA.
The specificity of the analytical method was checked by the chromatography of unfortified human urine samples which showed no other interfering peaks besides the analytes. Further, the sample solutions of 44 unfortified human urine samples containing residues of glyphosate were analysed in parallel using separation columns with phases of different selectivity. Analysed concentrations of glyphosate (n = 44 > LOQ) and AMPA (n = 25 > LOQ) ranged from 0.2 to 5 µg/L on both columns and correlated well: The respective slopes of the linear regression lines were close to unity (1.03 for glyphosate and 1.12 for AMPA) and the coefficients of determination (R2) reached satisfactory values (0.9968 for glyphosate and 0.9893 for AMPA). Therefore, it could be concluded that the primary transition was very selective for a reliable quantification of glyphosate and AMPA.

Basic calibration was performed by the measurement of 8 calibration solutions with concentrations ranging from 0.1 to 10 µg/L. A linear relationship between concentration and the ratio of the peak area of glyphosate and AMPA and its internal standards was observed. All calibration curve points were within 15% of their respective theoretical value. The linear correlation coefficients were typically > 0.99. Calibration curves for glyphosate and AMPA based on water and pooled human urine were both linear (each R2 > 0.99) and ran parallel. The slopes differed only by approximately 2%. This indicates that possible matrix effects are well compensated by the internal standards and matrix matched calibration solutions are not required for accurate determination of glyphosate and AMPA. The LOQ for glyphosate and AMPA was determined by fortification of human urine samples. The lower level at 0.1 µg/L demonstrated sufficient recovery (86 to 115%) and precision (8.9to 9.1%) for both analytes. This concentration was set as the LOQ of the GC-MS-MS method. The urine samples were analysed in a randomised order. Blank values (urine substituted by water) were measured during the analysis of urine samples regularly every 15th sample. All blank values were below the LOQ of 0.1 µg/L. Evaluation of the accuracy and precision of the method was performed through recovery experiments. Pooled human urine samples with no detectable amount of glyphosate and AMPA (each <0.1 µg/L) were fortified at 0.5, 1.0, 2.5, and 5 µg/L on 8 replicates each level. The recovery values ranged from 81 to 106% with a relative standard deviation (RSD) below 8.3%. Further, we performed recovery experiments using individual human urine samples to check for possible matrix effects caused by variations in the composition of the samples. Ten individual urine samples free of glyphosate and AMPA (each < 0.1 µg/L) were spiked at 0.5, 2.5 and 5 µg/L and were analysed in triplicate. The recoveries ranged from 87 to 110% proving that possible matrix effects were compensated by the internal standards $^{13}C_2$-^{15}N-glyphosate and ^{13}C-^{15}N-AMPA. In addition, the performance of the method was checked by measuring of control samples spiked at 0.5 and 2.5 µg/L during the analysis of the samples from this study (about every 33rd sample). A summary of the results of the control samples is provided in Table 4.

Table 6.9.8.6-4. Results of control samples concurrently analysed with the study samples

Analyte	Spiking level [µg/L]	Mean recovery [%]	Range [%]	RSD [%]	Number of samples
Glyphosate	0.5	103.0	84.4–113.3	8.6	15
	2.5	102.0	94.2–111.3	5.1	15
AMPA	0.5	102.1	82.4–112.4	9.4	15
	2.5	101.3	91.2–111.0	5.7	15

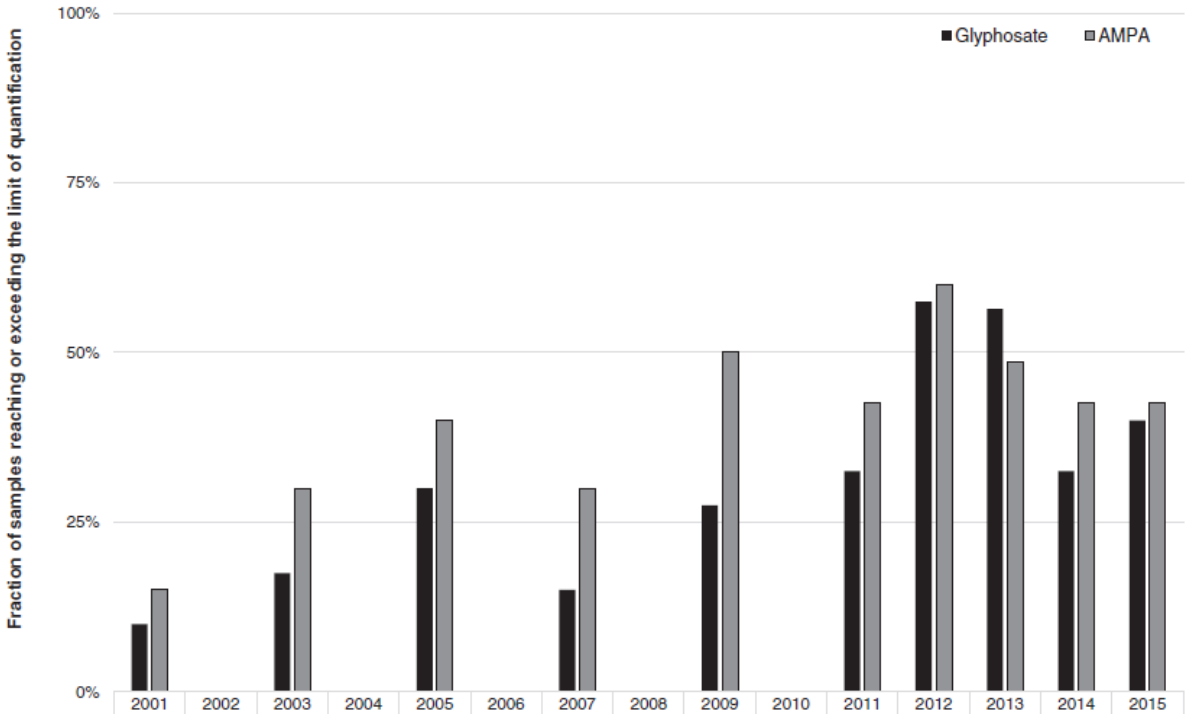
Statistical analysis; Glyphosate and AMPA concentrations below the LOQ were set to LOQ/2 prior to statistical evaluation. All data analyses were carried out in SPSS Statistics Version 20 (IBM Corporation, 2011). Differences between frequencies were tested with Pearson's Chi^2 test of independence after cross tabulation. Correlations between variables were quantified by Spearman's rank correlation coefficients, as concentration and other data mostly contained few extreme values. Box-plots were created in R Version 3.2.3 (R Core Team, 2015) displaying the 25th, median and 75th percentile as a box. The whiskers were set to extend to the minimum and maximum value, due to considerable skewness and obvious non-normality of the data. All p-values of 0.05 or lower were considered to indicate statistical significance.

Results

Urinary concentrations of glyphosate and AMPA in the main study sample

Frequency of quantifiable concentrations; Out of the 399 analysed urine samples, 127 (= 31.8%) contained glyphosate concentrations that reached or exceeded the LOQ of 0.1 µg/L. For AMPA this was the case for 160 (= 40.1%) of all samples. The fraction of samples at or above LOQ varied significantly over the years investigated, both for glyphosate ($p \leq 0.001$) and AMPA ($p = 0.005$). As displayed in Table 5 and Fig. 1, years with the highest quantification rates were 2012 (57.5%) and 2013 (56.4%) after rates having discontinuously increased from 10.0% in 2001. Fractions of at least 0.1 µg/L were lower again in 2014 and 2015 with 32.5% and 40.0%, respectively. The overall trend for quantifiable AMPA levels was quite similar. The highest fraction of samples reaching or exceeding the LOQ was observed for samples taken in 2012 (60.0%). The fractions of quantifiable levels of glyphosate and AMPA per year were generally higher in males. Especially for glyphosate, the principally increasing trend in urine concentrations was mainly due to samples provided by males (cf. Table 5).

Fig. 6.9.8.6-1. Temporal trend of glyphosate and AMPA in human 24 h-urine (fraction of samples at or above limit of quantification of 0.1 µg/L, ESB sampling location Greifswald , no self-reported specific dietary restriction)



	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Glyphosate	10.0%		17.5%		30.0%		15.0%		27.5%		32.5%	57.5%	56.4%	32.5%	40.0%
AMPA	15.0%		30.0%		40.0%		30.0%		50.0%		42.5%	60.0%	48.7%	42.5%	42.5%

Fractions of quantifiable glyphosate levels in samples from females were particularly high only in 2012 (55.0%) and 2013 (47.4%). The same difference between males and females was also apparent for AMPA, although the difference was less pronounced. Glyphosate sales in Germany have increased substantially from approximately 3300 t in 2000 to approximately 5400 t in 2014. The interim peak of approximately 7600 t in 2008 might be interrelated with the abolishment of EU set-aside requirements announced in 2007. Against the background of these data, the increase in quantifiable glyphosate and AMPA
concentrations in analysed ESB urine samples were in agreement with expectations. Although the internal exposure to glyphosate and AMPA seems to have decreased again since 2013, there was a clear increase in comparison to 2001. The possible reduction in exposure since 2013 indicated by ESB data may be due to changes in application of glyphosate in agriculture: Austria, for example, banned the pre-harvest use of glyphosate in 2013. Also in Germany, intended glyphosate uses as pre-harvest treatment have been restricted (e.g. to partial applications instead of whole field treatments) from 2014 onwards. Currently, no German sales data are available for the year 2015.

Distribution of concentrations; The 50th, 75th, and 95th percentiles and maximal values for glyphosate and AMPA levels by sex and study year are provided in Table 5. Only in 2012 and 2013 the median concentration of glyphosate was slightly above the LOQ of 0.1 µg/L. The 75th percentile exceeded the LOQ in all study years after 2007, reaching highest values in 2012 and 2013. The 95th percentiles of glyphosate concentrations in 24 h-urine were substantially higher in 2013 (1.25 µg/L) and 2014 (0.80 µg/L) compared to all other years. Also the maximum concentrations of glyphosate peaked in these two years (2013: 2.80 µg/L, 2014: 1.78 µg/L). The median urinary AMPA concentration only slightly exceeded the LOQ in 2012. With the exception of the first year of the study, 2001, all 75th percentiles exceeded the LOQ with the highest level observed in 2013. The 95th percentiles of AMPA levels peaked in 2013. The two highest AMPA concentrations were observed in samples from 2013 (1.88 µg/L and 1.54 µg/L). The observed urinary glyphosate and AMPA concentrations were in good agreement with findings from other studies. In view of these results, ESB data was considered to provide a reliable indication of the background exposure to glyphosate and AMPA in Germany and its change from 2001 to 2015. Comparability with other studies was limited, partly due to differences in the study population and in the type of urine samples.

Table 6.9.8.6-5. Summary statistics for glyphosate and AMPA concentrations in 24 h-urine samples (µg/L) by sex and year of sampling at ESB sampling location Greifswald (no self-reported specific dietary restrictions)

			Glyphosate					*AMPA*				
		N	% ≥ LOQ	P 50	P 75	P 95	Max.	% ≥ LOQ	P 50	P 75	P 95	Max.
2001	Male	20	15.0	<LOQ	<LOQ	0.26	0.40	15.0	<LOQ	<LOQ	0.25	0.29
	Female	20	5.0	<LOQ	<LOQ	<LOQ	0.11	15.0	<LOQ	<LOQ	0.21	0.22
	Total	40	10.0	<LOQ	<LOQ	0.12	0.40	15.0	<LOQ	<LOQ	0.22	0.29
2003	Male	20	20.0	<LOQ	<LOQ	0.25	0.37	40.0	<LOQ	0.14	0.18	0.18
	Female	20	15.0	<LOQ	<LOQ	0.16	0.20	20.0	<LOQ	<LOQ	0.19	0.20
	Total	40	17.5	<LOQ	<LOQ	0.16	0.37	30.0	<LOQ	0.13	0.18	0.20
2005	Male	20	40.0	<LOQ	0.14	0.26	0.26	45.0	<LOQ	0.19	0.24	0.24
	Female	20	20.0	<LOQ	<LOQ	0.19	0.24	35.0	<LOQ	0.13	0.26	0.29
	Total	40	30.0	<LOQ	0.11	0.25	0.26	40.0	<LOQ	0.17	0.24	0.29
2007	Male	20	10.0	<LOQ	<LOQ	0.20	0.26	35.0	<LOQ	0.14	0.23	0.23
	Female	20	20.0	<LOQ	<LOQ	0.14	0.14	25.0	<LOQ	<LOQ	0.19	0.20
	Total	40	15.0	<LOQ	<LOQ	0.14	0.26	30.0	<LOQ	0.13	0.21	0.23
2009	Male	20	40.0	<LOQ	0.11	0.22	0.30	55.0	0.11	0.18	0.55	0.81
	Female	20	15.0	<LOQ	<LOQ	0.12	0.12	45.0	<LOQ	0.16	0.20	0.20
	Total	40	27.5	<LOQ	0.10	0.13	0.30	50.0	<LOQ	0.17	0.26	0.81
2011	Male	20	50.0	<LOQ	0.14	0.38	0.51	60.0	0.13	0.22	0.48	0.65
	Female	20	15.0	<LOQ	<LOQ	0.11	0.11	25.0	<LOQ	0.11	0.32	0.37
	Total	40	32.5	<LOQ	0.11	0.25	0.51	42.5	<LOQ	0.18	0.34	0.65
2012	Male	20	60.0	0.12	0.22	0.48	0.57	65.0	0.15	0.21	0.61	0.66
	Female	20	55.0	0.11	0.16	0.44	0.63	55.0	0.11	0.19	0.46	0.50
	Total	40	57.5	0.11	0.20	0.48	0.63	60.0	0.12	0.21	0.56	0.66
2013	Male	20	65.0	0.18	0.29	0.55	0.63	60.0	0.18	0.35	1.03	1.54
	Female	19	47.4	<LOQ	0.16	2.80	2.80	36.8	<LOQ	0.16	1.88	1.88
	Total	39	56.4	0.11	0.27	1.25	2.80	48.7	<LOQ	0.29	1.54	1.88
2014	Male	20	55.0	0.11	0.20	1.12	1.78	60.0	0.13	0.19	0.25	0.26
	Female	20	10.0	<LOQ	<LOQ	0.63	1.15	25.0	<LOQ	<LOQ	0.60	0.97
	Total	40	32.5	<LOQ	0.11	0.80	1.78	42.5	<LOQ	0.16	0.25	0.97
2015	Male	20	70.0	0.16	0.22	0.45	0.49	50.0	<LOQ	0.18	0.38	0.41
	Female	20	10.0	<LOQ	<LOQ	0.37	0.57	35.0	<LOQ	0.13	0.38	0.39
	Total	40	40.0	<LOQ	0.16	0.45	0.57	42.5	<LOQ	0.16	0.38	0.41

Notes: N = sample size, LOQ = limit of quantification, P = percentiles, Max. = maximum value.

As displayed in Figs. 2 and 3 glyphosate and AMPA concentrations were generally higher in samples from male ESB participants compared to samples from female participants. From 2011 onwards, median levels and 75th percentiles for glyphosate were higher in males. Box-plots for AMPA concentrations showed the same pattern. The maximum values for glyphosate and AMPA concentrations in urine, however, were observed in samples from female ESB participants. The differences in urinary glyphosate might be due to differences in exposure patterns between males and females or to sex-related differences in physiological determinants of glyphosate and AMPA in urine.

Fig. 6.9.8.6-2. Box-plots of glyphosate concentrations in 24 h-urine samples by study year and sex (ESB sampling location Greifswald, no self-reported specific dietary restriction, concentrations below LOQ of 0.1 µg/L set to LOQ/2 = horizontal solid line, box displays 25th, median and 75th percentile, whiskers extend to minimum and maximum value)

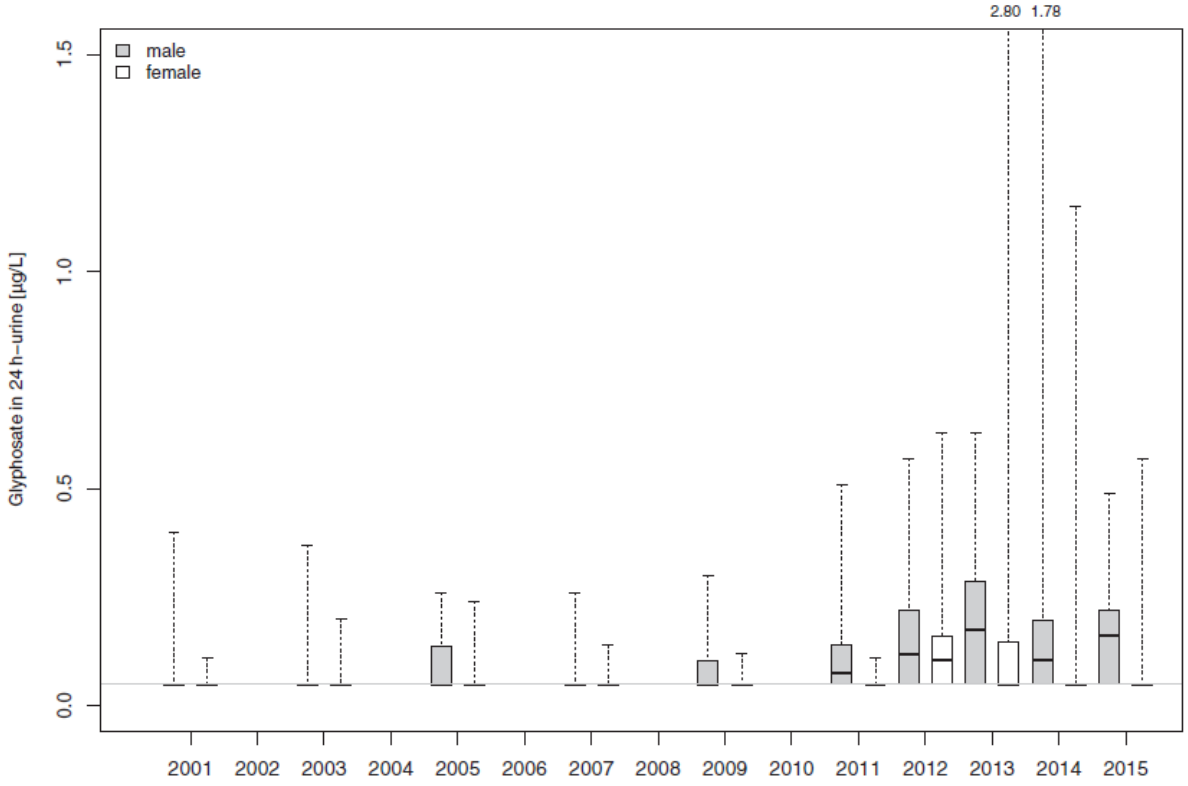
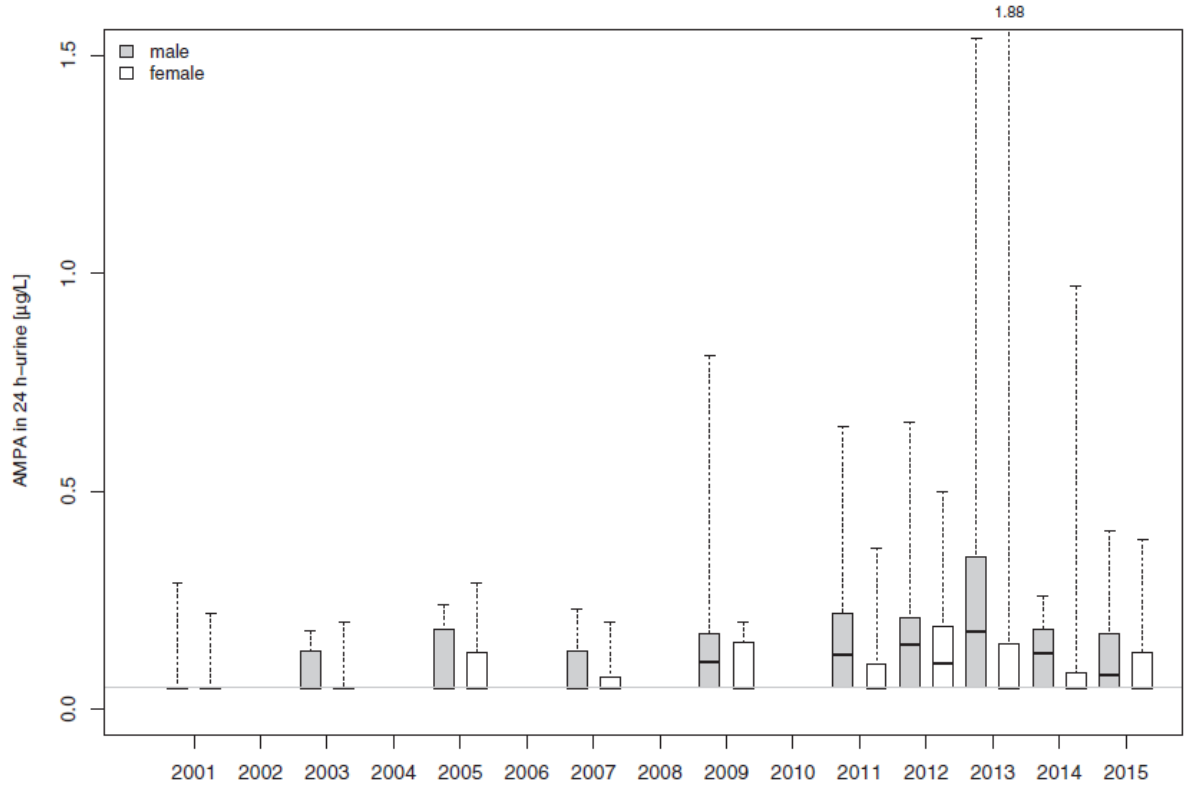

2.80 1.78
male
female
Glyphosate in 24 h−urine [µg/L]
0.0
0.5
1.0
1.5
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015

Fig. 6.9.8.6-3. Box-plots of AMPA concentrations in 24 h-urine samples by study year and sex (ESB sampling location Greifswald, no self-reported specific dietary restriction, concentrations below LOQ of 0.1 µg/L set to LOQ/2 = horizontal solid line, box displays 25th, median and 75th percentile, whiskers extend to minimum and maximum value



Correlations between glyphosate, AMPA and physiological parameters; Spearman rank correlations between glyphosate and AMPA levels in urine and physiological parameters observed in the main study sample are summarised in Table 6.

Table6.9.8.6-6. Spearman rank correlation coefficients between glyphosate and AMPA concentrations in 24 h-urine and physiological parameters

		AMPA in 24 h-urine [µg/L]	Body mass index [kg/m²]	Volume of 24 h-urine sample [mL]	Creatinine in 24 h-urine [g/L]
Glyphosate in 24 h-urine [µg/L]	*Corr. coeff.*	**0.506**	**0.161**	**−0.278**	**0.347**
	p-value	≤0.001	0.001	≤0.001	≤0.001
	N	399	399	398	398
AMPA in 24 h-urine [µg/L]	*Corr. coeff.*		0.079	**−0.327**	**0.373**
	p-value		0.114	≤0.001	≤0.001
	N		399	398	398
Body mass index [kg/m²]	*Corr. coeff.*			0.020	**0.252**
	p-value			0.692	≤0.001
	N			398	398
Volume of 24 h-urine sample [mL]	*Corr. coeff.*				**−0.760**
	p-value				≤0.001
	N				397

Notes: N = sample size, statistically significant correlation coefficients ($p \leq 0.05$) highlighted in bold

Glyphosate and AMPA concentrations in urine were statistically correlated (spearman rank correlations coefficient $r_S = 0.506$, $p \leq 0.001$). When calculating coefficients of rank correlation separately for each study year, glyphosate and AMPA levels correlated statistically significantly in all years except for the first two, 2001 and 2003. For the following eight years of the study, r_S ranged between 0.360 and 0.616 (all p-values ≤ 0.05). A statistically significant association between glyphosate and AMPA concentrations in urine was also observed

when cross tabulating all quantifiable and non-quantifiable levels for both analytes as well as when calculating the Pearson product-moment correlation coefficient (data not shown). There were, however, urine samples with comparatively high glyphosate and quite low AMPA concentrations, and vice versa. The coefficients of correlation of glyphosate and AMPA with BMI were comparatively low and statistically significant only for glyphosate. Correlations between BMI and glyphosate concentrations in urine were only statistically significant at the 5% level in 2011 (r_S = 0.344) and 2015 (r_S = 0.365). For AMPA, only the correlation of the concentrations in urine with the participants' BMI in 2015 reached statistical significance (r_S = 0.346). Glyphosate and AMPA concentrations in urine were consistently negatively correlated with the urine sample volume (r_S = -0.278 and -0.327) and positively correlated with urinary creatinine levels (r_S = 0.347 and 0.373). All these coefficients of correlation were statistically significant ($p \leq 0.001$). The BMI was positively correlated with the creatinine concentration in 24 h-urine samples (r_S = 0.252, $p \leq 0.001$). Glyphosate and AMPA concentrations in urine were positively associated with urinary creatinine in all study years. The coefficients of correlation were statistically significant at the 5% level in almost all study years. Examined for the individual years of the study, the r_S of urine sample volume and glyphosate as well as AMPA levels were consistently negative. The correlation, however, was often not statistically significant. These results warrant a further discussion on options for a combined consideration of glyphosate and AMPA in exposure assessment. The quite low, but statistically significant correlation between BMI and glyphosate deserves attention when further investigating glyphosate exposure via food consumption. The negative association of glyphosate and AMPA concentrations with 24 h-urine sample volumes and positive association with urinary creatinine concentrations were in line with expectations, as both parameters reflect the individual urinary diluteness. 24 h creatinine excretion was usually higher in males.

Comparison with other ESB sub-populations

To get a first insight into differences in exposures due to the place of residence and season of sampling, 40 urine samples collected in 2005 and 2015 at the ESB sampling location Muenster were also analysed for glyphosate and AMPA. In contrast to samples being taken in April/May in Greifswald, the annual Muenster sampling is carried out in January. The summary statistics for glyphosate and AMPA in this sub-population are given in Table 7.

Table 6.9.8.6-7. Summary statistics for glyphosate and AMPA concentrations in 24 h-urine samples (µg/L) by sex and year of sampling in two sub-populations from Muenster (no self-reported specific dietary restrictions) and Greifswald (self-reported vegetarians/vegans) analysed for comparison with the main study sample

			Glyphosate					*AMPA*				
		N	% ≥ LOQ	P 50	P 75	P 95	Max.	% ≥ LOQ	P 50	P 75	P 95	Max.
ESB sampling location Muenster (no self-reported specific dietary restrictions)												
2005	Male	20	0.0	<LOQ	<LOQ	<LOQ	<LOQ	30.0	<LOQ	0.12	0.20	0.22
	Female	20	10.0	<LOQ	<LOQ	0.34	0.54	25.0	<LOQ	<LOQ	0.28	0.30
	Total	40	5.0	<LOQ	<LOQ	<LOQ	0.54	27.5	<LOQ	0.11	0.24	0.30
2015	Male	20	15.0	<LOQ	<LOQ	0.23	0.31	30.0	<LOQ	0.11	0.34	0.45
	Female	20	15.0	<LOQ	<LOQ	0.17	0.17	40.0	<LOQ	0.17	0.28	0.31
	Total	40	15.0	<LOQ	<LOQ	0.17	0.31	35.0	<LOQ	0.15	0.28	0.45
Self-reported vegetarians/vegans (ESB sampling location Greifswald)												
2007	Male	0										
	Female	10	10.0	<LOQ			0.14	0.0	<LOQ			<LOQ
	Total	10	10.0	<LOQ			0.14	0.0	<LOQ			<LOQ
2015	Male	5	60.0	0.26			0.61	40.0	<LOQ			0.33
	Female	5	20.0	<LOQ			0.53	20.0	<LOQ			0.43
	Total	10	40.0	<LOQ			0.61	30.0	<LOQ			0.43

Notes: N = sample size, LOQ = limit of quantification, P = percentiles, Max. = maximum value.

In 2005 and 2015 the percentage of quantifiable glyphosate levels was significantly higher in the main study sample (Greifswald) than in Muenster (2005: 30.0% vs. 5.0%, p = 0.003 and 2015: 40.0% vs. 15.0%, p = 0.012). For AMPA no statistically significant differences between Greifswald and Muenster samples were observed in 2005 (40.0% vs. 27.5%, p = 0.24) and 2015 (42.5% vs. 35.0%, p = 0.49). Also the 75th and 95th percentile of urinary glyphosate concentrations in the main study sample were higher than in samples collected in Muenster. For AMPA these percentiles were quite similar for both populations. A second comparative subsample analysed for glyphosate and AMPA consists of 10 samples provided in 2007 and 2015 by self-reported vegetarians/vegans taking part in Greifswald (cf. Table 7). There was virtually no difference between self-reported vegetarians/vegans and the main study sample concerning quantifiable percentages of glyphosate in 2007 and

2015. For AMPA the fractions of samples with levels of at least 0.1 µg/L tended to be lower for vegetarians/vegans (2007: 0.0% vs. 30.0%, p = 0.047 and 2015: 30.0% vs. 42.5%, p = 0.47), being statistically significant only in 2007. In that year, all self-reported vegetarians/vegans who participated in Greifswald were female. When limiting the comparison to samples collected from women, the difference observed in 2007 was less pronounced and no longer statistically significant (0.0% vs. 25.0%, p = 0.083). Glyphosate concentrations in urine seem slightly higher in the main study sample in comparison to the Muenster sub-population. Although there were virtually no differences in urinary AMPA, this result indicated possible regional or seasonal differences in exposure. Against expectations, the results of this study did not considered to advocate urinary glyphosate and AMPA levels being higher in vegetarian/vegan participants. No equal sex distribution could be achieved for the sub-population of self-reported vegetarians/vegans, due to a low participation rate of male vegetarians/vegans. This might have reduced comparability of this sub-population, as males showed a tendency to exhibit higher glyphosate and AMPA concentrations in urine. Another limitation of this comparison was that vegetarian/vegan participants exhibit on average higher 24 h-urine sample volumes than in the main study sample without self-reported specifically restricted diet. In general, the sample sizes of the two sub-populations analysed for comparison were possibly too small to draw general conclusions on seasonal or regional effects and on effects of dietary preferences.

Health-relevance of observed internal exposure; The acceptable daily intake (ADI) for glyphosate derived by the European Food Safety Authority (EFSA) is 0.5 mg/kg/d (EFSA, 2015). Assuming a bodyweight of 60 kg, an oral absorption of 20% with fast elimination via urine, and a daily urine excretion of 1500 to 2000 mL, the concentration in 24 h-urine associated with this ADI resulted in 3000 to 4000 µg/L. This concentration was higher than the maximum concentration observed in this study (2.8 µg/L) by a factor of 1000. Considering EFSA's risk assessment, none of glyphosate concentration measured in ESB samples was considered problematic for human health. The International Agency for Research on Cancer (IARC), however, classified glyphosate in Group 2A ("probably carcinogenic to humans"; IARC,). Taking this assessment into account, especially the increasing trend in internal glyphosate exposure documented by ESB samples needs an attention with regard to human health.

Conclusion

Retrospective GC-MS-MS analyses of the general German population urinary samples collected during a period covering 2001 – 2015 revealed that 31.8% of analysed samples contained detectable level of glyphosate. For AMPA this was the case for 40.1% samples analysed. A peak of detectable glyphosate level was observed in 2012 (57.5%) and 2013 (56.4%), followed by a decrease in 2014 (32.5%) and 2015 (40.0%), which may be due to changes in glyphosate application in agricultural practice. Urinary glyphosate levels tended to be higher in males. Overall, the urinary level of AMPA showed a similar trend as glyphosate, with a statistically significantly correlation.

Assessment and conclusion by applicant:

The internal exposure levels of glyphosate and its main metabolite AMPA were analysed using the general German population urinary samples collected during a period covering 2001 – 2015 with similar sample sizes and sex distributions. Retrospective GC-MS-MS analyses revealed that 31.8% of analysed samples contained detectable level of glyphosate. For AMPA this was the case for 40.1% samples analysed. A peak of detectable glyphosate level was observed in 2012 (57.5%) and 2013 (56.4%), followed by a decrease in 2014 (32.5%) and 2015 (40.0%), which may be due to changes in glyphosate application in agricultural practice. Urinary glyphosate levels tended to be higher in males. Overall, the urinary level of AMPA showed a similar trend as glyphosate, with a statistically significantly correlation.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good monitoring study.

Reliability criteria of exposure studies by the applicant

Publication: Conrad *et al.* 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		

Reliability criteria of exposure studies by the applicant

Publication: Conrad *et al.* 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	Retrospective population monitoring study of glyphosate and AMPA in urine.
Test substance		
Exposure to formulations with only glyphosate as a.s.	NA	Exposure to glyphosate and AMPA mainly through the diet.
Exposure to formulations with glyphosate combined with other a.s.	NA	
Exposure to various formulations of pesticides	NA	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	General population.
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good monitoring study.		

Assessment and conclusion by RMS:
Agreed with the assessment and conclusion by the applicant. The study is considered reliable without restrictions. The study shows that from 2001 – 2015 31.8% of analysed samples contained detectable levels of glyphosate above the LOQ of 0.1 µg/L with a peak levels in 2012 and 2013. The 95th percentiles of glyphosate concentrations in 24 h-urine were substantially higher in 2013 (1.25 µg/L) and 2014 (0.80 µg/L) compared to all other years. Also the maximum concentrations of glyphosate peaked in these two years (2013: 2.80 µg/L, 2014: 1.78 µg/L). According to the study authors these highest urinary levels are a factor 1000 below the excretion that is expected when exposed at the ADI. Urinary levels of AMPA were correlated to glyphosate levels.

B.6.9.8.7. Literature study 7

Data point:	CA 5.9/007
Report author	Kongtip, P. *et al.*
Report year	2017
Report title	Glyphosate and Paraquat in Maternal and Fetal Serums in Thai Women
Document No	doi.org/10.1080/1059924X.2017.1319315 E-ISSN: 1545-0813
Guidelines followed in	None

study	
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Non-GLP
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

This longitudinal study measured the glyphosate concentrations found in maternal and umbilical cord serum in 82 pregnant women who gave birth in three provinces of Thailand. Through questionnaires and biological samples collected at childbirth, factors such as personal characteristics, family members occupation, agricultural activities, and herbicide use in agricultural work were evaluated as predictors of glyphosate levels in the pregnant women. Statistical analysis used univariate and binary multiple logistic regression, where the outcome was the probability of exposure to glyphosate above the limit of detection associated with occupation and household factors. The glyphosate concentrations in the pregnant women's serum at childbirth (median: 17.5, range: 0.2–189.1 ng/mL) were significantly higher (P < .007) than those in the umbilical cord serum (median: 0.2, range: 0.2–94.9 ng/mL). Women with glyphosate levels >limit of Detection (LOD) in serum at childbirth were 11.9 times more likely to report work as an agriculturist (P < .001), 3.7 times more likely to live near agricultural areas (P = .006), and 5.9 times more likely to have a family member who worked in agriculture (P < .001).

Materials and Methods

Subjects for a pilot birth cohort were recruited from pregnant women who came for prenatal care at three hospitals in Thailand: Amnatchareon Hospital in Amnatchareon Province in the northeast, Sawanpracharak Hospital in Nakhorn Sawan Province in the lower north, and Paholpolpayuhasena Hospital in Karnjanaburi Province in the west of Thailand, from May to December 2011. To be recruited, the women had to be in their 7th month of pregnancy, 19–35 years of age, not have diabetes or hypertension, and plan to give birth and have follow-up infant care at the recruiting hospital. The 82 subjects for this study included 81 full-term normal birth neonate and 1 full-term cesarean birth neonate.

During their 7th month of pregnancy, the women were interviewed about their general health, diet, and work exposures, including agricultural work, as well as about use of pesticides at home and work. Several questionnaires, each with several sections, constituted the data collection. The questionnaire was based on the type of information collected in previous studies of agricultural workers and modified for the conditions of agriculture and types of pesticide exposures experienced in Thailand. The questionnaires were reviewed by staff at each hospital, then piloted and revised based on comments. One questionnaire collected data on demographics and the mother's general health, whereas another collected birth data. The pesticide exposure questionnaire consisted of six sections; the first section contained 12 items related to pesticide use in the home or outside the home, as well as sources of drinking water. The second section contained 9 items about the woman's work history outside the home. The third section, with 10 items, covered agricultural activities if conducted by the woman. The fourth section, with 3 items, covered agricultural activities conducted by the woman during pregnancy. The fifth section, with 6 items, asked about the agricultural work of family members. The sixth section, with 30 items, collected detailed information only from those who were agricultural workers or had family members who were agricultural workers and included detailed information on the mixing and spraying of pesticides. To summarize, the pesticide exposure questionnaire had 40 items for all subjects and 30 items only for those who were self-identified as agricultural workers or who had family members who were agricultural workers. For comparisons with agriculturists, women with other occupations were used as the control group. The nurses at the prenatal clinics in the three study hospitals were trained to recruit and interview subjects. The maternal and umbilical cord serum was collected during delivery by the delivery nurses and was frozen at -45°C until analysis. This study was reviewed and approved by the Ethics Committee on Human Rights Related to Human Experimentation, Mahidol University, and the University of Massachusetts Lowell Institutional Review Board.

Glyphosate was obtained from Sigma-Aldrich Inc., Singapore. Acetonitrile, trimethylamine, methylene chloride, dichloromethane (all high-performance liquid chromatography [HPLC] grade), and sodium dodecyl sulfate (SDS) (gas chromatography [GC] grade) were purchased from Apex Chemical, Bangkok, Thailand. Other chemicals were of analytical grade. Analysis was performed with an HPLC system (Agilent 1200 Series) with a fluorescence detector for glyphosate. The serum sample was analysed on Luna 5 µm C18 (150 × 4.6 mm) column (Phenomenex, Torrance, CA, USA) with guard column at 45°C. A calibration curve was developed using hospital serum samples of non-subjects tested for the non-presence of glyphosate and then spiked with glyphosate to yield final concentrations of 12.5, 25, 50, 100, 150, and 200 ng/mL (n = 3 replicates). Samples of glyphosate were prepared using 250-µL of serum and 30 µL of derivatized glyphosate and injected into the HPLC. Evaluation of the detection limit was performed following the National Institute for Occupational Safety and Health method. For determination of accuracy and precision, concentrations of hospital serum samples of non-subjects tested for the non-presence of pesticide were used to prepare concentrations of 50, 100, and 150 ng of glyphosate/mL. Three replicates of each concentration were analysed on three separate days. The calibration curve for glyphosate was linear over the concentration range of 12.5–200 ng glyphosate/mL serum with the correlation coefficient of 0.998. The detection limit for the analysis of glyphosate in serum was 0.4 ng/mL. Values below the detection limit for serum glyphosate were used as the detection limit divided by 2 since the data were highly skewed. The recovery of the method ranged from 94.33% to 99.03% with a relative standard deviation (RSD) of <3% for glyphosate concentrations of 50–150 ng/mL.

The descriptive statistics were calculated using SPSS (SPSS version 18; PASW Statistics Base 18, Bangkok, Thailand). Since exposures were highly skewed due to the large percentage of values below the limit of detection (LOD), concentrations were reported as the median and range. For comparison of the paired mother and cord blood serum concentrations, only pairs with both measurements above the LOD levels were used with the Wilcoxon signed-rank test. Binary logistic regression was used to evaluate whether various factors were significantly associated with the probability of having glyphosate concentrations over the LOD. Several a priori factors were evaluated for inclusion as potentially significant covariates, such as maternal location/province and educational level of the mother, but these were found not to be significantly associated with the probability of having glyphosate concentrations over the LOD. Thus, occupational factors were evaluated in single-factor models and reported as unadjusted odds ratios (ORs). In models examining secondary exposure factors, maternal occupation was included as a covariate in the model to control for the influence of maternal agricultural work on serum levels.

Results

The average age of the 82 women who gave birth during the study was 26 years old (range: 19–34 years), with most having completed secondary school (41%), although 26% only completed primary school. Of the 82 women, 39% of the women described their occupation as agriculturist/farmer, whereas 21% listed themselves as a housewife, and about 13% were employees or owned their own business (often these are small retail stands with food or other items for sale). Of the 82 pregnant women who gave birth during the study, cord blood was only collected from 75 newborns, all born full term (37–41 weeks).

The percentage of maternal samples of glyphosate that were at or below the LOD was 46.3%, whereas for cord serum 50.7% were ≤LOD. Comparison of the glyphosate concentrations in paired serum samples of mother and cord blood that were both >LOD (n = 36) found that they were significantly different ($P < .001$), with the mother's serum levels the higher of the two. With regard to occupational factors predicting glyphosate exposures, the odds of having a detectable level of glyphosate in serum were 11.9 higher (95% confidence interval [CI] 3.6-39.5) than the LOD among women who worked in the fields compared with those who did not. Pregnant women who worked in agricultural fields during the first, second, or third trimester of pregnancy also had significantly elevated ORs of 13.5 (95% CI 2.8-64.3), 7.7 (95% CI 2.0-29.8), and 12.4 (95% CI 1.5-102.7), respectively, of having serum levels >LOD compared with those women who never worked in the fields. Likewise, pregnant women who reported picking crops during pregnancy had a significantly elevated OR (5.4; 95% CI 1.4-20.8)), whereas those who reported the agricultural activity of digging in farm soil or controlling weeds during pregnancy did not have elevated ORs.

With regard to secondary factors, pregnant women who lived near agricultural fields (<0.5 km) were significantly more likely to have serum glyphosate levels >LOD than those women living far from agricultural fields (OR=3.7, 95% CI 1.5-9.2). Note that there was no significant relationship between home location and agricultural occupation; thus, the adjusted OR did not reflect collinearity or confounding. Those pregnant women who reported a family member who was an agriculturist/farmer living in the same house were significantly more

likely to have elevated serum glyphosate than those who did not (OR=5.9; 95% CI 2.2-16.1); however, when adjusted for occupation, the OR became non-significant because of the high correlation between having a family member who was a farmer in the same house and maternal occupation as a farmer.

The most striking finding of this study was that glyphosate concentrations over the LOD were significantly more likely to be found in the serum of pregnant women collected at childbirth if their occupation was an agriculturist/farmer or conducted various agricultural tasks or if they lived near agricultural fields sprayed with pesticides (unspecified), when compared with women who had no agricultural exposures. Researchers measured glyphosate levels in urine among farmers in the United States and found that farmers who had skin contact with pesticides or did not use rubber gloves had significantly higher glyphosate concentrations in their urine. Among those who did not wear rubber gloves, appreciable differences were found in the urinary glyphosate levels between those who repaired equipment during applications or spilled during mixing/loading or application. This supports the notion that dermal contact is an important route of exposure for this herbicide. Researchers also reported urinary glyphosate in one of the children who lived 1.5 km away from the farm where their father sprayed glyphosate in France. However, in a US study, very few of the spouses (4%) and children (12%) who lived in a home within 1 mile of the sprayed farm fields had measurable urinary glyphosate on the day of spraying, and an even lower percentage had measurable levels on days 1–3 post application. Among the applicators, 60% had measurable glyphosate on the spraying day, with a decrease to 27% on the third day post application. This raises the question of the half-life of glyphosate and how the Thai agricultural women giving birth had measurable levels of glyphosate in their serum. Although these women had regular, repeated exposures to glyphosate, even during their final trimester of pregnancy, it is unlikely that they were exposed in the field on the day prior to giving birth. It is possible that these serum levels were caused by the drift of glyphosate spray, skin contact with the pesticide contaminated family member working in the fields, contamination of the home through clothing (take home exposure), or through poor storage of pesticides near the home. Alternatively, little is known about the metabolism and storage of pesticides during pregnancy and the impact on pesticide half-life. The median (range) glyphosate concentrations of maternal and umbilical cord serums in this current study were 17.5 (0.2–189.1) and 0.2 (0.2–94.9) ng/mL, respectively. The maternal serums of glyphosate at birth were significantly higher than those in cord blood serums. It is not known why maternal serum levels were higher than cord serum samples. At this time, only one study has looked at serum levels of pesticides in pregnant and non-pregnant Canadian women who were not agriculturists or living with a spouse who worked with pesticides. They found no detectable levels of glyphosate in the pregnant women before birth or in their cord blood samples. However, 5% (2/39) of the non-pregnant women in that study had measurable glyphosate serum levels (including one with a level of 93.6 ng/mL). Potential exposures in that study were assumed to be through consumption of pesticides associated with genetically modified foods. In our study, we found measurable glyphosate levels (>LOD) in 14.7% of the maternal serum samples of women who were not agriculturists/farmers by occupation. This is concerning, because researchers studied the effect of glyphosate exposures on the risk of miscarriage among women living on farms in Ontario, Canada, and found that women who were exposed to glyphosate before conception (from 3 months before up to conception) had a higher risk of spontaneous abortion.

Conclusion

This study suggests that agricultural activities do increase maternal serum levels of glyphosate even in samples taken on the day of birth. In the case of glyphosate, living near farmland where pesticides are sprayed can also significantly increase the risk of serum levels >LOD at birth. These results suggest that a study evaluating the long-term health of children exposed to herbicides during gestation should be considered. Given that herbicides make up the largest volume of pesticide imports in Thailand, and that imports continue to increase, further regulation of the sale and use of pesticides may help safeguard the health of Thai children.

Assessment and conclusion by applicant:

This study suggests that agricultural activities increase maternal serum levels of glyphosate, even in samples taken on the day of birth. Living near farmland where pesticides are sprayed can also significantly increase the risk of serum levels >LOD at birth. Limitations of this study include a small sample size (N=82) and large percentages of maternal samples cord serum that were at or below the LOD for glyphosate (46.3% and 50.7%, respectively).

This publication is considered relevant to the risk assessment of glyphosate but reliable with restrictions because the analytical method used for glyphosate could have been described in more detail.

Reliability criteria of exposure studies from the applicant

Publication: Kongtip *et al*., 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	NA	Subjects of a pilot birth cohort being monitored for glyphosate and paraquat.
Exposure to formulations with glyphosate combined with other a.s.	NA	
Exposure to various formulations of pesticides	NA	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	Analytical method for glyphosate could be described in more detail (derivatization).
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant to the risk assessment of glyphosate but reliable with restrictions because the analytical method used for glyphosate could have been described in more detail.		

Assessment and conclusion by RMS:

The RMS agrees with the conclusion by the applicant. The study is considered reliable with restrictions.

B.6.9.8.8. Literature study 8

Data point:	CA 5.9/008
Report author	McGuire M. K. *et al.*
Report year	2016
Report title	Glyphosate and aminomethylphosphonic acid are not detectable in human milk
Document No	doi: 10.3945/ajcn.115.126854 ISSN: 1938-3207
Guidelines followed in study	None
Deviations from current	NA

test guideline	
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	**Conclusion GRG:** Yes/Reliable without restrictions **Conclusion AGG:** The study is considered to be reliable without restrictions

Full summary of the study according to OECD format

It was sought to determine whether glyphosate and its metabolite aminomethylphosphonic acid (AMPA) could be detected in milk and urine produced by lactating women and, if so, to quantify typical consumption by breastfed infants. Milk (n = 41) and urine (n = 40) samples from healthy lactating women living in and around Moscow, Idaho and Pullman, Washington was collected. Milk and urine samples were analysed for glyphosate and AMPA with the use of highly sensitive liquid chromatography–tandem mass spectrometry methods validated for and optimized to each sample matrix. The milk assay, which was sensitive down to 1 µg/L for both analytes, detected neither glyphosate nor AMPA in any milk sample. Mean ± SD glyphosate and AMPA concentrations in urine were 0.28 ± 0.38 and 0.30 ± 0.33 µg/L, respectively. Because of the complex nature of milk matrixes, these samples required more dilution before analysis than did urine, thus decreasing the sensitivity of the assay in milk compared with urine. No difference was found in urine glyphosate and AMPA concentrations between subjects consuming organic compared with conventionally grown foods or between women living on or near a farm/ranch and those living in an urban or suburban non-farming area.

Materials and Methods

Human subjects – A total of 41 healthy lactating women living in and around Pullman, Washington, and Moscow, Idaho, were included in the study. To be eligible for participation, women had to be 1–3 months postpartum, breastfeeding and/or pumping milk 5 times/day and more, and aged 18 years and older. The exclusion criteria included current breast infection, use of antibiotics in the previous 30 days, and having an infant with signs or symptoms of illness in the previous 7 days to limit the subjects to healthy women nursing healthy infants. All but 1 subject also completed a 5-question survey documenting potential glyphosate exposure from the environment and diet.

Milk and urine collection and preservation - After cleaning the breast, approx. 30 mL milk was collected with an hospital-grade electric breast pump into a single-use sterile collection container. A midstream urine sample was also collected into a single-use sterile collection container. The sample containers were immediately placed in ice, separated into aliquots and frozen at -20^{0}C pending analysis. One subject failed to provide a urine sample.

Glyphosate and AMPA analyses - Milk and urine samples were analysed for glyphosate and AMPA using liquid chromatography-tandem mass spectrometry in the multiple reaction monitoring mode. Two precursor product ion transitions for each analyte and a stable isotope labelled internal standard for each analyte were used to ensure the selectivity of the analytical method. Although 2 quantitative precursor-product ion transitions were monitored, the results were reported using the most sensitive transition for each analyte. The assay was validated separately for milk and urine. Limits of detection (LOD) and quantification (LOQ) for glyphosate in milk were 1.0 and 10.0 µg/L, respectively. The LOD and LOQ for glyphosate in urine were 0.02 and 0.10 µg/L, respectively. The LOD and LOQ for AMPA in milk were 1.0 and 10.0 µg/L, respectively and those for AMPA in urine were 0.03 and 0.10 µg/L, respectively. Glyphosate and AMPA concentrations in milk were independently confirmed by another laboratory using the same liquid chromatography-tandem mass spectrometry method with minor modifications. Because of differences in instrumentation, the LODs with the more sensitive quantitative ion transitions were 6.0 and 9.0 µg/L for glyphosate and AMPA in human milk, respectively. The LOQ for human milk was 25.0 µg/L for both analytes. Duplicate aliquots from each milk sample were sent to each laboratory separately.

Statistical analyses - For glyphosate and AMPA concentrations in urine a generalized linear mixed model was used assuming a Poisson distribution with a logarithmic link function. For concentrations less than the respective LOD values, one-half LOD (0.01 and 0.015 µg/L for glyphosate and AMPA, respectively) nominal values were used for assessment. For concentrations between the LOD and LOQ, one-half LOQ (0.05 µg/L for both glyphosate and AMPA) nominal values were used for assessment. All values are presented as means ± SDs.

Results

Description of study population and glyphosate exposure - Women were aged 29 ± 5 years, 67 ± 17 days postpartum, and had a BMI (kg/m^2) of 26.8 ± 8.6. 75% of them lived in an urban or suburban non-farming region of the Palouse, and 58% of them reported that they made no effort to eat foods characterized as organic, although they sometimes included them in their diets for convenience. 15% reported ever having personally mixed or used any type of weed killer. All but one of the women having reported ever doing so had mixed or used a weed killer containing glyphosate. In general, subjects were highly educated Caucasian women who participated in the study during either the summer or winter months.

Glyphosate and AMPA concentrations in milk - Regardless of where the samples were analysed, none of the milk samples contained detectable amounts of either glyphosate or AMPA.

Glyphosate and AMPA concentrations in urine - Glyphosate was detectable in nearly all (*n* =37) of the urine samples and was quantifiable in 29 of them. Glyphosate values ranged from below the LOD (<0.02 µg/L) to 1.93 µg/L, with a mean concentration of 0.28 ± 0.38 µg/L. AMPA was also detectable in nearly all (*n* = 38) of the urine samples and quantifiable in 29 of them. Urine AMPA values ranged from below the LOD (<0.03 mg/L) to 1.33 µg/L, with a mean concentration of 0.30 ± 0.33 µg/L. There were no statistically significant effects of consuming organic compared with conventional foods or living on/near a farm compared with living in an urban/suburban region on concentrations of glyphosate in urine, respectively. Neither were there statistically significant effects of consuming organic compared with conventional foods or living on/near a farm compared with living in an urban/suburban region on concentrations of AMPA in urine. Adjusting for potential covariates (age, time postpartum, BMI, parity) did not alter these conclusions. When raw, untransformed values were used in the analysis, there was a statistically significant positive correlation between urinary glyphosate and AMPA concentrations.

Discussion and Conclusion

The results provide evidence that the concentrations of glyphosate and AMPA in milk produced by healthy women are below the detection limits of available validated analytical assays. In urine, glyphosate and AMPA were detectable in many samples, but the concentrations were very low (<0.02 to 1.93 and <0.03 to 1.33 µg/L, respectively) and well below values reported in other healthy adult populations. The RfD for glyphosate is 1.75 mg/kg bw/day. US EPA considers AMPA to be of similar or lesser toxicity than glyphosate and determined that it should be exempt from regulation regardless of concentrations observed in food or feed. Thus, a woman with a typical weight for the study participants, 75 kg, could consume as much as 131.25 mg glyphosate/day with no expected negative effects. Taking an oral bioavailability of 20% into account and assuming that all glyphosate absorbed is excreted within 24 hours and all absorbed glyphosate is excreted in urine, the urinary output would be 26,250 µg/day. In the current study, the highest reported glyphosate concentration in urine was 1.93 µg/L. As such, even allowing for a relatively high urine output (3 L/day), the highest glyphosate excretion in our study would be 5.79 µg/day, a value which is more than 4,500 times lower. Applying similar parameters and logic to a 5-kg infant with a mean milk intake of 0.7 L/day and a milk glyphosate concentration of 1 µg/L (the LOD value), then the maximum daily consumption of glyphosate would be 0.7 µg/day which is more than 12,000 times lower than the RfD. It is important to emphasize that the larger international study from which these samples originate was not designed to detect small differences in urine glyphosate and AMPA concentrations based on dietary choices, location of residence (e.g., urban compared with rural), or occupational glyphosate exposure. Detecting small-effect sizes at statistically significant concentrations and adequate statistical power would require 4–5 times as many observations than used in this study.

Assessment and conclusion by applicant:

In this study breast milk and urine samples from lactating women were analysed for glyphosate and AMPA. The results provide evidence that the concentrations of glyphosate and AMPA in milk produced by healthy women are below the detection limits of available validated analytical assays. In urine, glyphosate and AMPA were detectable in many samples, but the concentrations were very low and well below the values reported in other healthy adult populations.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with all the reliability criteria of an exposure study.

Reliability criteria from the applicant for exposure studies

Publication: McGuire *et al*., 2016	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.		
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Reference material (glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides	Y	Exposure mainly via food
Study		
Study design clearly described	Y	Monitoring of glyphosate in urine and breast milk of lactating women
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with all the reliability criteria of an exposure study.		

Assessment and conclusion by RMS:
Agreed with the assessment and conclusions by the applicant. The study is considered reliable without restrictions.
The results provide evidence that the concentrations of glyphosate and AMPA in milk produced by healthy women are below the detection limits of available validated analytical assays. In urine, glyphosate and AMPA were detectable in many samples, but the concentrations were very low (<0.02 to 1.93 and <0.03 to 1.33 µg/L, respectively) and well below values reported in other healthy adult populations.

B.6.9.8.9. Literature study 9

Data point:	CA 5.9/009
Report author	Sierra-Diaz, E. *et al.*
Report year	2019
Report title	Urinary pesticide levels in children and adolescents residing in two agricultural communities in Mexico
Document No	doi:10.3390/ijerph16040562 ISSN: 1660-4601
Guidelines followed in study	None.
Deviations from current	Not applicable

test guideline	
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG:** Yes/Reliable with restrictions **Conclusion AGG:** The study is considered to be with restrictions

Full summary of the study according to OECD format

The objective of this study was to measure the concentration and prevalence of pesticides in a cross-sectional study involving children and adolescents under 15 years of age in two small farming communities located in in State of Jalisco in Mexico, with both communities having very similar characteristics.

Urine samples (first-morning urine) were taken from children under 15 years of age in Agua Caliente located near the largest lake in Mexico (n = 192) and in Ahuacapán located in the south coast of the state (n = 89). A total of 281 urine samples obtained in both communities and processed for the determination of pesticides with high-performance liquid chromatography together with tandem mass spectrometry.
In 100% of the samples, at least two pesticides of the 17 reported in the total samples were detected. The presence of glyphosate was detected in more than 70% of the cases. The mean urinary level of glyphosate 0.363 ± 0.3210 ng/mL in Agua Caliente and 0.606 ± 0.5435 ng/mL in Ahuacapán, detected in 72.91% and 100% of their respective total samples. Substantial differences were detected regarding the other compounds.

Materials and methods

A cross-sectional study was carried out simultaneously in two communities in State of Jalisco, which is one of the three states in Mexico with the greatest index of people poisoned due to the application of pesticides. The first of these was, Agua Caliente, near Lake Chapala, the largest lake in Mexico, and the second was a community in the region of the south coast of the state (Ahuacapán).

Beginning hundreds of years ago, multiple autochthonous communities of people of Nahua native origin who were dedicated to fishing and agriculture settled on the bank of Lake Chapala. Since 2016, the Department of Public Health of the University of Guadalajara has carried out studies in the zone, specifically in the community of Agua Caliente, Poncitlán Municipality, State of Jalisco. In this community, health problems have been detected such as malnutrition and albuminuria, specifically in children and adolescents under the age of 17 years.

The community is inhabited by 998 persons, whose main activities are farming (37.9%), construction work (29.3%), and laboring 7.2% and who alternate with fishing as a means of subsistence. The most common local crops are corn, seasonal beans, and chayote (*Sechium edule*), which the inhabitants irrigate with lake water. The weekly average family income is approximately 52.63 USD.

The community of Ahuacapán is found toward the coast of the Pacific Ocean, in a 180 km straight line from Agua Caliente. A total of 950 inhabitants live there and their principal economic activity is agriculture, producing sugar cane, corn, tomatoes, citrus, and horticultural products. The water necessary for agriculture derives from springs, deep wells, and the Ayuquila River. The weekly family income in this community is approximately 75 USD. In both localities, there is a total of 550 children and adolescents (58%) aged between less than 1 year and 15 years.

For the urine sampling, communities were invited to participate voluntarily, with the approval of the Department of Public Health of the University of Guadalajara and the local authorities of the two communities. The sample included only children and adolescents aged under 17 years in Agua Caliente and those under 12 years of age in Ahuacapán. Their parents were informed concerning the objective of the study and, after obtaining the parents' signed consent, the minors were asked for a urine sample (first-morning urine). In both communities, anthropometric measurements (weight and height) were performed on the minors.

Urine samples were transported to the laboratory and were processed for the determination of pesticides with the HPLC/MS/MS (high-performance liquid chromatography coupled with tandem mass spectrometry) method with Agilent Technologies® Model 1200 equipment for HPLC and Model 6430B for MS/MS spectrometry. The method for HPLC used a column Zorbax Eclipse XDB C18, Rapid Resolution, 2.1 × 50 mm, 3.5 µm. Mobile

phases: A, 0.1% formic acid in water; B, acetonitrile (ACN); gradient of 40% to 100% B; injection volume, 5 µL; flow, 0.5 min; curve range for each pesticide, 0.01 to 1000 µg/mL. The latter was performed at the Laboratory of Applied Pharmacokinetics of the University Center of Exact and Engineering Sciences of the University of Guadalajara. With this method, it was possible to determine the presence of 16 pesticides, as presented in Table 1.

Table 6.9.8.9-1. Category of pesticides analysed in urine samples from the two communities.

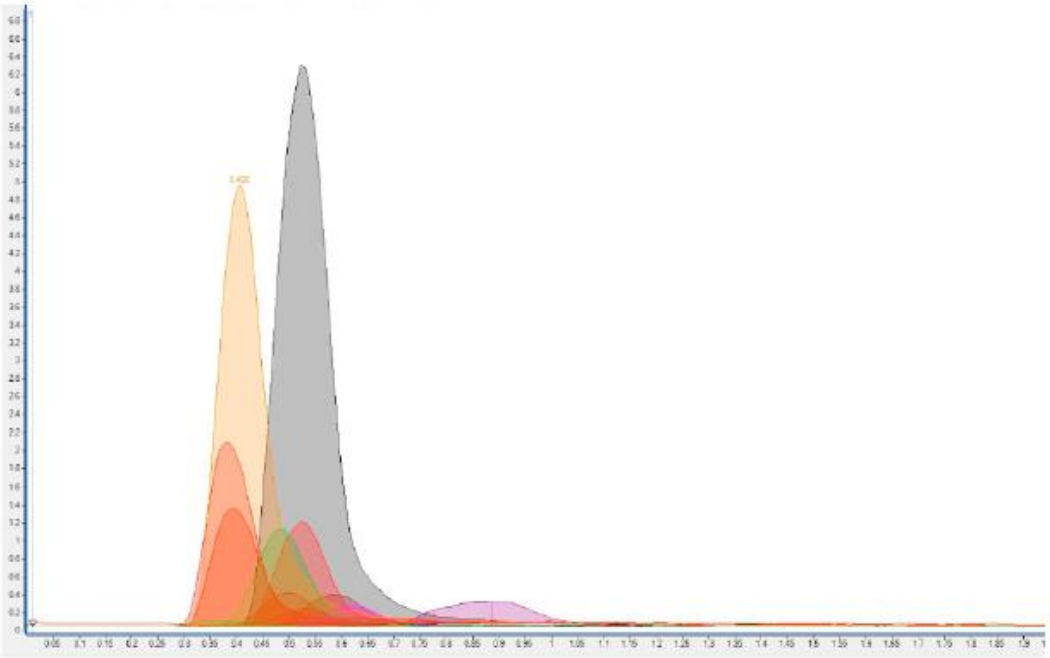
Name	IUPAC ID	PubChem CID	Agrochemical Category
Acetochlor	2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide	1988	Herbicide
Atrazine	6-chloro-4-N-ethyl-2-N-propan-2-yl-1,3,5-triazine-2,4-diamine	2256	Herbicide
Carbendazim	methyl N-(1H-benzimidazol-2-yl)carbamate	25429	Fungicide
Carbofuran	(2,2-dimethyl-3H-1-benzofuran-7-yl) N-methylcarbamate	2566	Insecticide, Nematicide, Acaricide
Cyhalothrin	[cyano-(3-phenoxyphenyl)methyl] 3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate	5281873	Insecticide
Diazinon	*O,O*-Diethyl O-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl] phosphorothioate	3017	Insecticide, Acaricide
Dimethoate	2-dimethoxyphosphinothioylsulfanyl-N-methylacetamide	3082	Insecticide, Acaricide
Emamectin	4″-Deoxy-4″-epi-methylamino-avermectin B1; Epi-methylamino-4″-deoxy-avermectin	11549937	Insecticide
Enilconazole (imazalil)	1-[2-(2,4-dichlorophenyl)-2-prop-2-enoxyethyl]imidazole	37175	Fungicide
Glyphosate	2-(phosphonomethylamino)acetic acid	3496	Herbicide
Malathion	diethyl 2-dimethoxyphosphinothioylsulfanylbutanedioate	4004	Insecticide, Acaricide
Methomyl	methyl (1E)-N-(methylcarbamoyloxy)ethanimidothioate	5353758	Insecticide
Metoxuron	3-(3-chloro-4-methoxyphenyl)-1,1-dimethylurea	29863	Herbicide
Molinate	S-ethyl azepane-1-carbothioate	16653	Herbicide
Pyraclostrobin	Methyl N-[2-[[1-(4-chlorophenyl) pyrazol-3-yl]oxymethyl]phenyl]-N-methoxycarbamate	6422843	Fungicide, plant growth regulator
Thiabendazole	4-(1H-benzimidazol-2-yl)-1,3-thiazole	5430	Fungicide

Conditions for MS/MS spectrometry are described in Table 2 and Figure 1 shows a chromatogram urine sample.

Table 6.9.8.9-2. Mass spectrometer conditions for pesticide determination.

Mass spectrometer conditions				
Electrospray Interface Condition				
Gas emperature	350 °C			
Gas flow	12 L/min			
Nebulizer	25 psi			
Capillary	+4000	−4000		
Compound name	**Precursor Ion**	**Product Ion**	**Fragmentor**	**Polarity**
L-Cyhalotrin (225.1)	467.1	225.1	80	Positive
Meclizina (201.1)	391.2	201.1	90	Positive
Pyraclostrobin (163)	388	163	120	Positive
Malation (99)	331	99	80	Positive
Clorpyrifos (200)	325	200	30	Positive
Oxandrolona (289.2)	307.2	289.2	100	Positive
Oxandrolona (271.2)	307.2	271.2	100	Positive
Oxandrolona (229.1)	307.2	229.1	100	Positive
Diazinon (153)	305	153	160	Positive
Imazalil (159)	297	159	160	Positive
Paration (264)	292	264	90	Positive
Paration (236)	292	236	90	Positive
Acetoclor (224.2)	270.1	224.2	60	Positive
Acetoclor (148.4)	270.1	148.4	60	Positive
Picloram (222.9)	240.9	222.9	90	Positive
Picloram (194.9)	240.9	194.9	90	Positive
Dimethoate (171)	230	171	80	Positive
Metoxuron (72.1)	229.1	72.1	93	Positive
Ametryn (186)	228.1	186	120	Positive
Ametryn (96)	228.1	96	120	Positive
Carbofuran (123)	222	123	120	Positive
Atrazine (132)	216	132	120	Positive
Thiabendazole (131)	202	131	120	Positive
Carbendazim (160)	192.1	160	110	Positive
Molinate (55.1)	188.1	55.1	78	Positive
Methomyl (106)	163.1	106	30	Positive
Methomyl (88.1)	163.1	88.1	30	Positive
Methomyl (65)	163.1	65	30	Positive
Emamectina (158.1)	887.1	158.1	60	Positive
Glyphosate (149.9)	168	149.9	80	Negative
Glyphosate (124.2)	168	124.2	80	Negative
2,4-D (161.1)	219	161.1	50	Negative

Figure 6.9.8.9-1. Shows a chromatogram of a urine sample.



Statistical analysis

For the statistical description, absolute frequencies, percentages, means, and standard deviations (SD) were used. Statistical significance was evaluated by means of the Mann–Whitney U, the Chi-squared, and the Fisher exact tests. To compare the two populations and evaluate the differences in the urine pesticide levels, the Mann–Whitney U test was used. Similarly, to compare the frequency of detection rate, the Fisher test was used. Statistical significance was considered with a p of ≤ 0.05. For data processing, Excel® (Microsoft, Redmond, WA, USA) and Epi Info ver. 7.2 (Centers for Disease Control and Prevention (CDC) Atlanta, GA, USA) statistical software were used.

This research was carried out with the authorization of the ethics committee of the Department of Public Health of the University of Guadalajara (registration number DCSP/CEI/2016/260618/038).

Results

A total of 281 children participated, of whom 192 (68.3%) corresponded to the community of Agua Caliente with an average age of 9.4 years (range, 5–15 years). In the community of Ahuacapán, 89 (31.7%) samples were collected, with an average age of 8.31 years (range, 5–13 years; Table 3).

Table 6.9.8.9-3. Demographic and anthropometric data of the children in both communities.

Variable	Agua Caliente (n = 192)	Ahuacapán (n = 89)
Gender		
Female	84 (43.8%)	40 (44.9%)
Male	108 (56.3%)	49 (55.1%)
Age (years)	9.40 (SD 2.52)	9.31 (SD 2.05)
Age groups		
5–8 y	78 (40.6%)	49 (55.1%)
9–11 y	67 (34.9%)	34 (38.21%)
12–15 y	47 (24.5%)	6 (6.7%)
Weight (kilograms)	29.39 (SD 10.06)	32.27 (SD 11.73)
Height (centimeters)	131.58 (SD 14.12)	132.94 (SD12.97)
Body mass index (k/m^2)	16.46 (SD 2.44)	17.72 (SD 3.66)

Detection of the pesticides was frequent in both communities. Substantial differences in exposure and detection rate were also identified (Table 4). Glyphosate was detected with a higher frequency in Ahuacapán than in Agua

Caliente, which presented in 100% of the minors studied in Ahuacapán vs. 73% in Agua Caliente ($p > 0.001$), with minimal values of 0.0020 ng/mL and maximal values of 2.63 ng/mL. Glyphosate is currently one of the most utilised herbicides in Mexico. The differences between the two localities were considered to be associated with the agro-industrial activity and the practices that have become generalised among the small producers of basic crops.

In general terms, 100% of the study subjects were exposed to at least two of the compounds identified in urine. Positive results are noteworthy of six of the compounds in more than 70% of the subjects studied in both communities: malathion, metoxuron, glyphosate, dimethoate, enilconazole, and acetochlor. The greatest prevalence was for the herbicides (60.49%), in second place, fungicides (39.05%), and lastly, the insecticides (20.92%).

The urinary samples in this study was collected during winter, during which there is a significant diminution in the agricultural use of pesticides. However, the authors emphasised that consumption of a conventional diet with seasonal products could play a very important role regarding the presence of these pesticides in urine.

Table 6.9.8.9-4. Frequencies, percentages, means, and standard deviations (SD) of pesticides in urine.

Pesticide	Agua Caliente	Ahuacapán	
	n (%)	*n* (%)	*p* (Fisher Test)
	Mean ng/mL (SD)	Mean ng/mL (SD)	*p* (Mann–Whitney)
Acetochlor	161 (83.85) 0.008 (0.0867)	44 (49.43) 0.001 (0.0017)	<0.01 0.04
Atrazine	22 (11.45) 0.016 (0.0486)	22 (24.71) 0.043 (0.0930)	<0.01 0.06
Carbendazim	29 (15.10) 0.141 (0.4192)	52 (41.57) 0.330 (0.5040)	<0.01 <0.01
Carbofuran	1 (0.52) 0.246	0	NA NA
Cyhalothrin	138 (71.87) 0.083 (0.0823)	45 (50.56) 0.080 (0.0855)	<0.01 0.52
Diazinon	29 (15.10) 0.007 (0.0199)	20 (22.47) 0.008 ± 0.0180	0.09 0.41
Dimethoate	179 (93.22) 0.146 (0.1834)	44 (49.43) 0.169 (0.2299)	<0.01 0.03
Emamectin	6 (3.12) 0.006 (0.0339)	9 (10.11) 0.019 (0.0582)	0.02 0.34
Enilconazole	177 (92.18) 1.582 (5.6623)	29 (32.58) 0.069 (0.1023)	<0.01 <0.01
Glyphosate	140 (72.91) 0.363 (0.3210)	89 (100) 0.6060 (0.5435)	<0.01 <0.01
Malathion	191 (99.47) 0.681 (0.6431)	55 (61.79) 0.177 (0.1730)	<0.01 <0.01
Methomyl	46 (23.95) 0.016 (0.0292)	0	<0.01 <0.01
Metoxuron	188 (97.91) 0.038 (0.0403)	50 (56.17) 0.037 (0.0414)	<0.01 0.10
Molinate	79 (41.14) 0.191 (0.3698)	55 (61.79) 0.273 (0.4240)	<0.01 <0.01
Pyraclostrobin	62 (32.29) 0.049 (0.2331)	37 (41.57) 0.042 (0.0509)	0.08 0.18
Thiabendazole	29 (15.10) 0.007 (0.0511)	24 (26.96) 0.002 (0.0046)	<0.01 0.12

Conclusion

In a comparative cross-sectional study in two agricultural communities with very similar characteristics in Mexico (a total of 281 children participated in the study), glyphosate was detected in more than 70% of the cases

in both communities, having a higher prevalence rate along with malathion, metoxuron, compared to other pesticides analysed in the study. The mean urinary levels of glyphosate were 0.363 ± 0.3210 ng/mL in Agua Caliente and 0.606 ± 0.5435 ng/mL in Ahuacapán. In general, substantial differences in exposure and detection rate were also identified between the communities.

Assessment and conclusion by applicant:

In a comparative cross-sectional study using the urine of children living in two agricultural communities with very similar characteristics in Mexico (a total of 281 children participated in the study), glyphosate was detected in more than 70% of the cases in both communities. The mean urinary levels of glyphosate were 0.363 ± 0.3210 ng/mL in Agua Caliente and 0.606 ± 0.5435 ng/mL in Ahuacapán.
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no validation data were presented for the analytical method employed.

Reliability criteria of exposure studies by the applicant

Publication: Sierra-Diaz *et al*., 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides	Y	
Study		
Study design clearly described	Y	Survey of glyphosate concentrations in children.
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	?	No pesticide exposures reported.
Sampling scheme sufficiently documented	Y	Production of one early morning urine sample.
Analytical method described in detail	Y	
Validation of analytical method reported	N	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no validation data were presented for the analytical method employed.		

Assessment and conclusion by RMS:

Agreed with the conclusion and assessment by the applicant. The study is considered reliable with restrictions.

In a comparative cross-sectional study using the urine of children living in two agricultural communities, the presence of glyphosate was detected in more than 70% of the cases. The mean urinary level of glyphosate were 0.363 ± 0.3210 ng/mL in Agua Caliente and 0.606 ± 0.5435 ng/mL in Ahuacapán, detected in 72.91% and 100% of their respective total samples.

B.6.9.8.10. Literature study 10

Data point	CA 5.9/010
Report author	Steinborn, A. *et al.*
Report year	2016
Report title	Determination of Glyphosate Levels in Breast Milk Samples from Germany by LC-MS/MS and GC-MS/MS
Document No.	doi.org/10.1021/acs.jafc.5b05852 E-ISSN: 1520-5118
Guidelines followed in study	Guidance document on analytical quality control and validation procedures for pesticide residues in food and feed, SANCO/12571/2013
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No/Not stated
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

This study describes the validation and application of two independent analytical methods for the determination of glyphosate in breast milk. They are based on liquid chromatography − tandem mass spectrometry (LC-MS/MS) and gas chromatography − tandem mass spectrometry (GC-MS/MS), respectively. For LC-MS/MS, sample preparation involved an ultra filtration followed by chromatography on an anion exchange column. The analysis by GC-MS/MS involved an extraction step, clean-up on a cation exchange column, and derivatization with heptafluorobutanol and trifluoroacetic acid anhydride. Both methods were newly developed for breast milk and are able to quantify glyphosate residues at concentrations as low as 1 ng/mL. The methods were applied to quantify glyphosate levels in 114 breast milk samples, which had been collected from August to September of 2015 in Germany. The mothers participated at their own request and thus do not form a representative sample. In none of the investigated samples were glyphosate residues above the limit of detection found.

Materials and Methods

Chemicals – Glyphosate standard solution (10 µg/mL), reference glyphosate and internal standard ($^{13}C_2{}^{15}N$ labeled glyphosate) were purchased from LGC Standards, Wesel, Germany and from Dr. Ehrenstorfer, Augsburg, Germany.

Collection of breast milk samples - Breast milk samples were collected in August and September 2015 by the Governmental Institute of Public Health of Lower Saxony, Germany and by the Bavarian Authority for Health and Food Safety Germany for the analysis of glyphosate. All participants signed a declaration of consent concerning the use of their samples for scientific purposes. Participating mothers had not been selected by random sampling and thus do not form a representative sample. There were no restrictions relating to, for example, age and point of sampling during the lactation period for participating in the monitoring program. The milk samples for this study were collected and stored in polypropylene tubes which remained frozen during storage and shipment. In total, 114 milk samples were analysed and the participants completed a self-

administered questionnaire. Information on sample collection, biometric data and self-reported pesticide exposure of the participants is given in Table 1.

Table 1: Biometric data of study participants

parameter	samples from Bavaria, Germany	samples from Lower Saxony, Germany
number of samples	17	97
age of mother (years)		
median	32.1	32.0
range	26–39	22–39
body weight of mother (kg)		
median	63.0	67.0
range	54–90	48–102
duration of lactating period (weeks)		
median	11.0	18
range	3–80	4–52
self-reported exposure to pesticides	6 participants	32 participants

The questionnaire also asked for the place of residence and the jobs practiced in the last 10 years. Thirty-eight participants declared the use of chemical insecticides, herbicides or wood preservatives. At least one participant has worked in a residue analytical laboratory and used pesticide standards regularly. Twenty of the 114 breast milk samples were divided each into two subsamples to allow the parallel analysis by LC-MS/MS and GC-MS/MS.

Fortification of breast milk samples for performance tests - A homogeneous sample of breast milk was prepared and spiked with different volumes of a glyphosate standard solution in water at 10 µg/mL. Twenty-eight stored breast milk samples from a previous study of the Governmental Institute of Public Health of Lower Saxony were pooled and 4 aliquots of 100 mL were spiked with glyphosate resulting in concentrations of 0.5, 1, 3, and 5 ng/mL. An additional aliquot of the pooled sample served as the control. All performance samples were divided into two subsamples and analysed in parallel with LC-MS/MS and GC-MS/MS. These samples served as independent quality control samples.

Sample Preparation for LC-MS/MS analysis - Removal of fat by centrifugation and removal of proteins by ultra-filtration in one step through centrifugal filtration using a molecular weight cutoff filter of 30 kDa was found to be suitable. To 3 mL of sample, 30 µL of internal standard solution containing 1000 ng $^{13}C_2{}^{15}N$ glyphosate/mL was added to obtain a concentration of 10 ng/mL. After mixing, the sample was transferred to the top part of the cutoff filter tube. The filter was centrifuged at 3500 rpm for 20 minutes and 500 µL of the filtrate was then transferred to the LC filter vial, the solution was filtered and the vial used for measurement. After this procedure, one mL of final extract contained the glyphosate residue of one mL breast milk.

LC-MS/MS analysis - The LC-MS/MS system used consisted of a Nexera UHPLC system from Shimadzu equipped with a 5500 Qtrap system (Sciex) in triple-quad mode. The LC system consisted of an anion exchange LC column (Dionex Ionpac AS 11 (2 × 250 mm) and a AG-11 guard column (2 × 50 mm) from Thermo Fischer Scientific). Twenty-five µL of standard solution or filtrate were injected into the LC system and glyphosate was eluted from the column using a gradient of water (A) and 1 mM citric acid solution brought to a pH of 11 by addition of a dimethyl amine solution (B). Gradient elution consisted of following steps: 100% A from 0 to 2 minutes, linear to 25% B in 5.5 minutes, and linear to 50% B in 2.5 minutes which was held for 4 minutes. After returning to 100% A in 0.5 minutes the system was re-equilibrated for 7.5 minutes before the next injection. The total run time (including injection) was 22.5 minutes with a flow rate of 0.4 mL/min. A column temperature of 40°C was maintained while the temperature of the samples in the autosampler was 12°C. All transitions were measured using a declustering potential of -75 V, an entrance potential of -10 V and a dwell time of 50 ms. The Turbospray source was used in negative electrospray mode using the following parameters: Curtain gas 20 arbitrary units, collision gas medium, ion spray - 4000 V, temperature 400 °C, ion spray gas 1: 40 and ion spray gas 2: 50 arbitrary units. The mass transitions for evaluation [m/z] were 168.2 → 62.8 for quantification, 168.2 → 79.0 for confirmation and, 171.2 → 62.8 for $^{13}C_2$ ^{15}N glyphosate.

Sample Preparation for GC-MS/MS analysis - A 2 mL milk sample was extracted with 3.75 mL of 0.6% acetic acid and centrifuged for 5 minutes at 4000 rpm. Two mL of the supernatant liquid was extracted with 2 mL dichloromethane for 2 minutes and the two phases separated by centrifugation at 4000 rpm for 5 minutes. One mL of the supernatant liquid was filtered using a 0.45 µm Nylon filter and a cation exchange clean-up was performed using disposable Bio-Rad Poly-Prep columns filled with 1.72 g (equivalent to 2 mL filling volume) of AG 50W-X8 resin (H^+-form). A 0.55 mL aliquot of the filtered extract (corresponding to 0.2 mL breast milk) and 0.100 mL of internal standard solution (20 ng/mL) were added onto the cation exchange column and were eluted followed by 2.0 mL of CAX solution (800 mL HPLC grade water, 13.5 mL 10 N HCl solution and 200 mL methanol). Both eluates were discarded. Glyphosate residues were eluted from the column with 12.5 mL of CAX solution and evaporated to dryness. The residues were dissolved in 1.0 mL of CAX solution. Derivatization reagent (2,2,3,3,4,4,4-hepta fluoro-1-butanol and trifluoroacetic acid anhydride 1:1) was cooled to a temperature of - 20°C. 0.05 mL of the dissolved eluate (corresponding to 0.01 mL breast milk) was added to 1.5 mL of the chilled reagent and after 5 minutes derivatization is started by heating to 92-97°C for 1 hour. After cooling, the excess of derivatization reagent was removed by evaporation. The dry residue was dissolved in 0.2 mL of ethyl acetate containing 0.2 mL/L citral and then concentrated to a final volume of 20 µL. Citral was used to prevent adsorption of the analytes in the inlet and the GC column. Following this procedure, one mL of final extract contained the glyphosate residue of 0.5 mL breast milk.

GC-MS/MS analysis - The GC-MS/MS system consisted of a Thermo Trace GC Ultra equipped with a TriPlus liquid autosampler, split/splitless injector and MS detector TSQ Quantum with triple quadrupole (Thermo Fisher Scientific). The GC column was a Optima 5HT of 30 m length and 0.25 mm internal diameter coated with a 0.25 µm film (Macherey-Nagel). Four µL of the extracts were injected splitless with the injector temperature at 280 °C. The oven temperature was held at 80°C for 1.5 minutes, ramped up at 10 °C/minute to 180 °C, then ramped up at 30°C/minute to 300 °C and held at 300 °C for 2.8 minutes. The carrier gas was helium and the flow rate 1 mL/minute. The expected retention time for the glyphosate derivative was 9.1 minutes. The temperature of the ion source was 280 °C and the electron impact (EI) energy was 70 eV with an emission current of 50 µA. Mass transition for evaluation [m/z] was 612 → 213 for quantification, 611 → 261 for confirmation, and 615 → 213 for $^{13}C_2$ ^{15}N glyphosate. Calculations were performed using the ratio of the peak areas of the quantifier transition of glyphosate derivative and the internal standard derivative. Calibration solutions were prepared by volumetric dilution of a glyphosate stock solution in a solution containing 20 ng/mL internal standard. The dilutions were made in CAX solution. Aliquots of 0.05 mL of these calibration solutions were derivatized as described for the breast milk extracts. The concentration of the derivatized calibration solutions ranged from 0.01 to 10 ng/mL. The concentration of the internal standard in the final extract was always 5 ng/mL.

Results and Discussion

Since a very low transfer was observed of glyphosate into muscle, milk and fat in farm animal metabolism studies the LOQ should be as low as possible. The LC-MS/MS method was validated for glyphosate in accordance with the requirements of the EU guidance document for quality control and validation procedure. Recovery and precision of glyphosate were determined for 6 or 7 replicates at two fortification levels. The linearity of the system was tested by injecting 8 standards in water in a concentration range from 0 to 50 ng/mL. A linear relationship between concentration and the ratio of the peak area of glyphosate and its internal standard was observed with a coefficient of determination greater than 0.99. All calibration points were within 20% of the theoretical value. Quantification was performed using single-point calibration which is acceptable if the response of the analyte in the samples is close to the response in the standard. The average recovery of the LC-MS/MS method was 99% at 1 ng/mL and 91% at 5 ng/mL with a relative standard deviation of 16% and 7%, respectively. The LOQ of the LC-MS/MS method of 1 ng/mL demonstrated sufficient recovery and precision. Possible matrix effects were corrected using the stable isotope labeled internal standard $^{13}C_2{}^{15}N$ glyphosate. At a concentration of 0.5 ng/mL, a signal-to-noise ratio of approximately 4 was obtained. This concentration is considered the LOD of the LC-MS/MS method.

For the analysis of glyphosate by GC-MS/MS, extraction with acidified water was combined with clean-up on a cation exchange column to remove interfering substances present in breast milk. To enable analysis by gas chromatography glyphosate was derivatized using heptafluoro-1-butanol and trifluoroacetic acid anhydride. Calibration was performed with freshly prepared derivatives of 8 glyphosate standard solutions in the concentration range from 0.01 to 10 ng/mL. All standard solutions contained the internal standard at 5 ng/mL and the coefficient of determination was always equal to or greater than 0.9980. The average recovery of the GC-MS/MS method was 84% at 1 ng/mL and 83% at 10 ng/mL with a relative standard deviation of 13% and 8%, respectively. All results obtained by GC-MS/MS had to be corrected for (derivatization reagent) blank

interferences. A set of at least 4 reagent blanks were analysed within each set of breast milk samples. The average measured blank values ranged from 0.2 to 0.6 ng/mL. The relative standard deviations of blank values in the sample sets ranged from 19% to 33%. Considering the blank values from the derivatization reagent, the LOQ of the GC-MS/MS method was 1 ng/mL. Notwithstanding the interference problem of the GC-MS/MS method, both analytical methods were able to measure glyphosate residues in breast milk with an LOQ of 1 ng/mL.

In total, 114 different breast milk samples were analysed for glyphosate. Seventy-five samples were analysed by LC-MS/MS only. Because of the lower performance, only 19 samples were analysed exclusively by GC-MS/MS. Twenty milk samples were analysed by both methods. In addition to the 114 samples, 5 samples for performance testing were analysed by both LC-MS/MS and GC-MS/MS, 4 breast milk samples which were spiked in advance with glyphosate and one control sample. Glyphosate was identified by LC-MS/MS in all samples spiked with glyphosate. The recoveries for the LC-MS/MS method were 110%, 97% and 102% for the spiking levels of 1, 3, and 5 ng/mL, respectively. In the sample spiked at 0.5 ng/mL, glyphosate could still be detected by the LC-MS/MS method. Due to the interference problem in GC-MS/MS method, no clear detection of glyphosate was possible at this level.
The recoveries for the GC-MS/MS method were 70%, 70%, and 54% for the spiking levels of 1, 3, and 5 ng/mL, respectively. Generally, the GC-MS/MS method tended to result in lower concentrations, probably due to the correction for derivatization reagent blank values. The higher bias of the GC-MS/MS method might be due to dilution steps using very small volumes. The concentration step to yield the final volume might have resulted in a partial loss of the glyphosate derivative. Nevertheless, both methods are able to quantify glyphosate residues in breast milk at or above a concentration of 1 ng/mL. Because of the lack of significant blank values in the LC-MS/MS method, residues of glyphosate higher than 0.5 ng/mL are still detectable by this method. In none of the 114 breast milk samples obtained from German women glyphosate was detected.

Conclusion

A LC-MS/MS and a GC-MS/MS method were developed for the detection and quantification of glyphosate in human breast milk. Both methods have been fully validated and are suitable for the determination of glyphosate with an LOQ of 1 ng/mL. The LC-MS/MS method allows the detection of glyphosate at or above a level of 0.5 ng/mL. The LC-MS/MS method is much faster than the GC-MS/MS method, thus making it suitable for higher sample throughput. The positive findings of glyphosate in breast milk of American women could not be confirmed by the results of this study. In none of the 114 breast milk samples collected from German women in August and September 2015 glyphosate was found within the detection limitations of the analytical methods. Available data from farm animal studies on glyphosate with non-labeled material support these results. They provide no indication of a significant carry-over into fatty tissues or milk even at high dosing levels.

Assessment and conclusion by applicant:
Two analytical methods were developed for the determination of glyphosate in human breast milk. In the first method fat was removed by centrifugation and the proteins by ultra-filtration using a molecular weight cutoff filter of 30 kDa. The final extract was then analysed by LC-MS/MS. In the second method the milk sample was acidified with acetic acid, centrifuged and the supernatant extracted with dichloromethane. The aqueous phase was filtered and cleaned-up using a cation exchange resin. The final extract was then analysed by GC-MS/MS after derivatization with heptafluoro-1-butanol and trifluoroacetic acid anhydride. $^{13}C_2{}^{15}N$ glyphosate was used as the internal standard in both methods. Both analytical methods were validated according to the EU guidance document on analytical quality control and validation procedures for pesticide residues in food and feed (SANCO/12571/2013) and were found suitable for the determination of glyphosate in human breast milk with an LOQ of 1 ng/mL. In August and September 2015, 114 breast milk samples were collected from German women and were analysed for glyphosate. In none of the samples analysed glyphosate concentrations were found at or beyond the LOQ.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the breast milk samples analysed were collected on a voluntary basis and there were no restrictions for participating in the monitoring program. As a consequence the samples cannot be considered representative of the German population. Both analytical methods developed were validated in accordance with the EU guidance on the procedures for the analysis of pesticide residues in food and feed.

Reliability criteria of exposure studies from the applicant

Publication: Steinborn et al., 2016.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	Y	Guidance document on analytical quality control and validation procedures for pesticide residues in food and feed, SANCO/12571/2013.
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.		Self-reported exposure to pesticides.
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides		
Study		
Study design clearly described	Y?	Mainly the development of methods for the analysis of glyphosate in human breast milk and applied to a set of 114 samples from German women.
Population investigated sufficiently described	Y?	Biometric data of the study population were rather limited. The samples were collected on a voluntary basis and without restrictions.
Exposure circumstances sufficiently described	Y?	No detail was provided on self-reported exposure to pesticides.
Sampling scheme sufficiently documented	Y?	More detail could have been provided.
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	None of the 114 samples analysed was positive for glyphosate.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the breast milk samples analysed were collected on a voluntary basis and there were no restrictions for participating in the monitoring program. As a consequence the samples cannot be considered representative of the German population. Both analytical methods developed were validated in accordance with the EU guidance on the procedures for the analysis of pesticide residues in food and feed.		

Assessment and conclusion by RMS:

Agreed with the assessment and conclusions by the applicant. The study is considered reliable with restrictions.

In all of the 114 breast milk samples analyzed, the glyphosate concentrations were at or below the LOQ of 1 ng/mL.

B.6.9.8.11. Literature study 11

Data point:	CA 5.9/011
Report author	Trasande, L. *et al.*
Report year	2020
Report title	Glyphosate exposures and kidney injury biomarkers in infants and young children
Document No	doi.org/10.1016/j.envpol.2019.113334 E-ISSN: 1873-6424
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Yes/Reliable without restriction **Conclusion AGG:** The study is considered to be without restrictions

Full summary of the study according to OECD format

The goal of this study was to assess biomarkers of exposure to glyphosate and assess potential associations with renal function in children. While previous studies have indicated that glyphosate may have nephrotoxic effects, few have examined potential effects on kidney function in children. In this study, three cohorts across different phases of child development and measured urinary levels of glyphosate. Associations of glyphosate with three biomarkers of kidney injury was evaluated: albuminuria (ACR), neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury marker 1 (KIM-1). Multivariable regression analyses examined associations of glyphosate with kidney injury biomarkers controlling for covariates. Glyphosate was identified in 11.1% of the total participants. The herbicide was detected more frequently in the neonate population (30%). Multivariable regression models failed to identify significant associations of log-transformed glyphosate with any of the kidney injury biomarkers, controlling for covariates age, sex, and maternal education. While detectability of glyphosate in children's urine at various ages and stages of life was confirmed, there was no evidence for renal injury in children exposed to low levels of glyphosate.

Materials and methods

Study populations; We briefly describe a longitudinal birth cohort of children (Starting Early), in whom urine samples were measured at 10-19 months of age, as well as two cross-sectional studies Preventing Environmental Exposures in Pregnancy (PEEPS), and Bright Start which represent older children (ages 3-8) and newborns (<30 days), respectively. The three cohorts are comprised of chronologically ordered categories of age in children as follows: Bright Start (newborns), Starting Early, and PEEPS. Inclusion in the present study was determined if sufficient urine was available for the experimental testing. All research was performed in accordance with relevant guidelines/regulations. In particular, informed consent was obtained from participants' parents or their legal guardians for use of collected samples for use in future studies such as this one of glyphosate exposure.

Urine collection and storage; Urine samples for each cohort were obtained at study visits using age-appropriate methods (urine bags and cotton balls in untrained babies and infants, freshly voided urine in trained children) and immediately transferred to sterile polyethylene cups. Within 2 h after collection from the infants, urine was transferred to cryovials for storage at -80 °C prior to laboratory analysis. We measured urinary markers of kidney injury NGAL, and KIM1 using Luminex xMAP technology (see below). Urine albumin and creatinine were measured using standard methods of quantitative spectrophotometry at the ARUP National Reference

Laboratory (Salt Lake City, Utah) and the results were used to calculate ACR (Albumin-to-Creatinine Ratio [mg:mg]).

Analysis of urine samples for glyphosate; 200 µL of urine sample was transferred into a 15 mL polypropylene (PP) tube and spiked with the labeled internal standard mixture (2-^{13}C, 99%; ^{15}N, 98+% Glyphosate), allowed to stand at room temperature for 30 min and then diluted to 1.0 mL (5-fold dilution) with 1% formic acid in water. The diluted sample was vortexed for 1 min, centrifuged and filtered through a nonsterile regenerated cellulose (RC) membrane filters (0.2 mm; Phenomenex, Inc., Torrance, CA, USA). The filtrate then transferred into an auto sampler vial for LC-MS/MS analysis. An Agilent 1260 Series HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) coupled with an ABSCIEX 4500 QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA) running under negative mode electrospray ionization was used for the analysis. An anion-exchange column, Dionex IonPac AS 21 (2 mm x 250 mm, 7 µm) was employed for the separation of target chemicals under isocratic elution condition with a mobile phase consist of 1% formic acid in water and acetonitrile (95:5) mixture. Isotopic dilution mass spectrometry with MRM mode of analysis was used for selective quantification of the target chemicals. The QA/QC protocols included matrix spike (mean spike recoveries (n=5) for glyphosate was 109.5%) and procedural blanks (non-detected or < LOQ). Midpoint calibration standard and HPLC grade water were injected between every 20 samples analysed to check the instrument detection linearity and carry-over effects, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as three and ten times of signal to noise ratio, respectively. LOD for glyphosate was 0.1 ng/mL, and LOQ was 0.33 ng/mL.
Analysis of kidney injury biomarkers; NGAL was measured in human urine using the Luminex performance human kidney Biomarker kit (FCSTM16-01, R&D Systems). Urine samples were thawed and diluted 1:10 in calibrator diluent and protocol followed manufacturer's instructions. Data were captured on a Luminex MAGPIX instrument with xPonent 4.2 software. Standard curve R^2 values were 0.98 and high- and low-quality controls were within expected range. KIM-1 was measured in human urine using the Milliplex MAP Human Kidney Injury Magnetic Bead Panel Kit (HKI1MAG-99K, EMD Millipore). Urine samples were thawed and diluted 1:2 in assay buffer and protocol followed manufacturer's instructions. Data were captured on a Luminex MAGPIX instrument with xPonent 4.2 software. Standard curve R2 values were 0.97 and high- and low-quality controls were within the expected range.

Statistical analysis; We first described the demographics of our study population (N = 108), including age, sex, maternal education, race/ethnicity, BMI category, and intervention arm in the case of the Starting Early group. Body mass index (BMI), calculated as weight in kilograms divided by height in meters squared, was used to measure adiposity in the Starting Early group. We used standardized BMI *z* scores given that BMI varies widely by age and sex, following the Centers for Disease Control and Prevention year 2000 norms. Overweight and obese were categorized as BMI *z*-score of 1.036 or greater (85th percentile for age and sex) and 1.64 or greater (95th percentile), respectively. We calculated median glyphosate, albuminuria, KIM-1, and NGAL levels, as well as interquartile ranges for all biomarkers and percent detected for glyphosate levels by study group. We also described mean and standard deviation in each one of the mentioned biomarkers of interest in the total population. Univariate regressions examined log-transformed glyphosate concentrations and their detectability (using logistic regression) as dependent variables with covariates examined singly. We created dummy variables for maternal education with one category for those children whose mothers did not complete a high school education and another for those whose mothers at least completed high school, and created a dummy variable for missing values (N = 34) that we used as reference. We also created an ordinal variable reflecting the age of the children in increasing order from youngest to oldest type as follows: Healthy Start, Starting Early, and PEEPS (N = 108). We also performed univariate analyses by study arm in the Starting Early sample (N = 66). Multivariable analyses of continuous log-transformed glyphosate exposure examined age, sex, and maternal education as predictors (N = 108). We assessed differences in detectability of glyphosate using multivariable logistic regressions with age, sex, and maternal education as predictors. All regressions examining predictors of log-transformed glyphosate were retransformed from the logarithmic scale. We then examined associations of glyphosate with kidney injury. First, we calculated Pearson correlation coefficients for all log-transformed kidney injury markers KIM-1, NGAL, and ACR, as well as log-transformed glyphosate levels. For glyphosate levels below the limit of detection (LOD), we imputed values equal to LOD/square root of 2. We also performed univariate and multivariable regressions of urinary log-transformed glyphosate as predictor of log-transformed kidney injury markers KIM-1, NGAL, and ACR, controlling for age, sex and maternal education. Sensitivity analyses were also included; we performed univariate and multivariable regressions of creatinine-adjusted urinary log-transformed glyphosate as predictor of log-transformed kidney injury markers KIM-1, NGAL, and ACR. Statistical analysis was performed with Stata/IC Version 14 (StataCorp: College Station, TX).

Results

The population was largely Hispanic, and comprised roughly equal numbers of males and females (Table 1). Only 4% of our participants had a mother with a college degree but the majority of parents in our study population graduated from high school (62.1%). The mean urinary glyphosate concentration was 0.278 ± 0.228 µg/mL, with a range of 0.105-2.125 ng/mL. We identified glyphosate in 11.1% of the participants. The herbicide was detected in 7.6-30% of the three cohorts: 30% of neonates had glyphosate exposure above the LOD, followed by PEEPS cohort (12.5%) and Starting Early (7.6%). We also observed a wide detection range in NGAL biomarkers. We also noted that 40% of children in the Starting Early cohort were overweight or obese. Younger children had higher urinary levels of glyphosate in both univariate and multivariable regressions. Children with mothers without a high school diploma had slightly lower levels of urinary glyphosate relative to those with mothers who attained a high school education in our multivariable regression. However, we found no other significant associations of demographic covariates with glyphosate in univariate or multivariable models. Similarly, we found no statistically significant associations of demographic factors in relationship to detectability of urinary glyphosate in children. No univariate regressions with urinary glyphosate identified a statistically significant association with increased renal biomarker injury (Fig. 1A-C respectively and Table 2). Multivariable regressions with urinary glyphosate failed to identify statistically significant associations of log-transformed glyphosate with KIM-1, NGAL, or ACR (Table 2). In sensitivity analyses of univariate and multivariable regressions with creatinine-adjusted log-transformed glyphosate, there was also no statistically significant evidence for increased levels of markers for renal injury (Table 2). In additional sensitivity analyses without the newborns similar results were reported with no statistically significant associations of log-transformed glyphosate with kidney injury biomarkers (Table 2).

Table B.6.9.8.11-1: Study population (n=108)[a]

Demographics		N	%
Male (Sex)		56	51.9
Study	Bright Start (Neonates)	10	9.3
	Starting Early	66	61.1
	Intervention Arm	39	59.1
	Control Arm	27	40.9
	PEEPS	32	29.6
Maternal Education	None	7	9.5
	Elementary/Middle	21	28.4
	High School/General Equivalency Diploma	35	47.3
	Some college	7	9.5
	College grad	3	4.1
	Other	1	1.4
Race/Ethnicity	Hispanic	67	90.5
	Non-Hispanic White	5	6.8
	Non-Hispanic Black	1	1.4
	Non-Hispanic Asian	1	1.4
BMI Categories[b]	Underweight	2	3.0
	Normal	40	60.6
	Overweight	12	18.2
	Obese	12	18.2

Distribution of Glyphosate and Kidney Injury Biomarkers		
	Mean (SD)	Detection Range (Min. & Max. Concentrations)
Glyphosate (ng/mL)	0.278 (0.228)	0.105–2.125
KIM-1 (ng/mL)	80.17 (76.92)	12.38–560.6
NGAL (pg/mL)	12,486 (21,934)	530.7–131,196
ACR[c] (mg/g)	26.23 (25.43)	1.414–119.0

Distribution of Glyphosate and Kidney Injury Biomarkers by Study Groups (N = 108)			
Study	Healthy Start (Neonates)	Starting Early	PEEPS
Glyphosate (ng/mL), Median (IQR)	<LOD (<LOD-1.06)	<LOD (<LOD-LOD)	<LOD (<LOD-LOD)
Percent Detected	30.0%	7.58%	12.5%
KIM-1 (ng/mL), Median (IQR)	88.9 (71.0–114)	47.1 (33.8–71.7)	76.5 (40.3–140.2)
NGAL (pg/mL), Median (IQR)	3770 (2520–7200)	9080 (3880–18,700)	1920 (1240–5770)
ACR[c] (mg/g), Median (IQR)	9.45 (6.00–34.3)	28.0 (19.1–42.4)	5.66 (3.54–9.00)

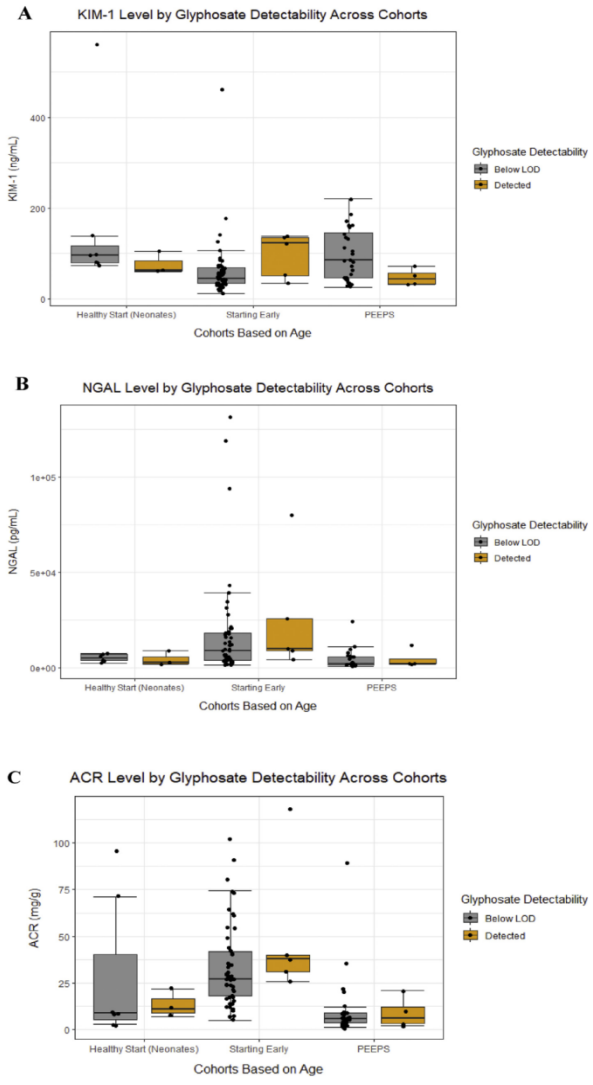
[a] Maternal Education 34 missing, Race/Ethnicity 34 missing, BMI 42 missing, KIM-1 9 missing, NGAL 13 missing, and ACR 12 missing.
[b] Underweight (Below −1.036 SD); Normal (Between −1.036 and + 1.036 SD); Overweight (Between + 1.036 and + 1.64 SD); Obese (Above +1.64 SD).
[c] Albumin-to-creatinine ratio.

Table B.6.9.8.11-2: Multivariable regressions of creatinine-adjusted urinary log-transformed glyphosate as predictor of log-transformed KIM-1, NGAL, ACR biomarkers[a]

All ages N = 108 [a]			
Increment per one log unit increase	KIM-1	NGAL	ACR
Glyphosate Univariate	0.974 (−17.92, 27.78)	−663.8 (−2462, 2641)	−0.287 (−6.773, 10.91)
Glyphosate Multivariable	−1.55 (−14.93, 17.62)	−887.8 (−1800, 662.2)	−5.05 (−14.14, 9.85)
Creatinine-adjusted models[b]			
Glyphosate Univariate	−1.648 (−15.22, 17.47)	−640.5 (−3242, 4079)	7.603 (−9.522, 32.88)
Glyphosate Multivariable	−3.70 (−13.47, 10.26)	−819.8 (−1688, 657.8)	0.858 (−13.80, 22.43)
Non-neonates N = 98[c]			
Increment per one log unit increase	KIM-1	NGAL	ACR
Glyphosate Univariate	−5.351 (−24.13, 27.39)	1489 (−4383, 17944)	3.002 (−10.66, 35.99)
Glyphosate Multivariable	−3.568 (−14.90, 15.96)	395.3 (−5128, 12782)	12.05 (−37.98, 106.6)
Creatinine-adjusted models[d]			
Glyphosate Univariate	−4.89 (−20.66, 22.43)	2083 (−5679, 23234)	8.24 (−16.60, 54.17)
Glyphosate Multivariable	−3.73 (−14.45, 14.88)	132.8 (−5165, 11668)	10.37 (−25.33, 72.33)

[a] KIM-1 N = 99; NGAL N = 95; ACR N = 96. Multivariable regression models controlling for demographic covariates age, sex, and maternal education, where maternal education missing observations were imputed as the majority category "Equal or Greater Than High School".
[b] Creatinine-adjusted regression models KIM-1 N = 95; NGAL N = 92; ACR N = 96.
[c] KIM-1 N = 89; NGAL N = 86; ACR N = 86. Multivariable regression models controlling for demographic covariates age, sex, and maternal education, where maternal education missing observations were imputed as the majority category "Equal or Greater Than High School".
[d] Creatinine-adjusted regression models KIM-1 N = 85; NGAL N = 83; ACR N = 86.

Figure B.6.9.8.11-1: A. The dot plot illustrates the urinary KIM-1 level in participants enrolled in the different studies, 1B. The dot plot illustrates the urinary NGAL level in participants enrolled in the different studies, 1C. The dot plot illustrates the urinary albumin:creatine ratio (ACR) in participants enrolled in the different studies.



Discussion

The main findings of this study are the substantial and frequent prevalence of glyphosate in children across the first decade of life in three independently assembled samples. We identified glyphosate in 11.1% of the total participants, and the herbicide was detected more frequently in the neonate population (30%). However, urinary concentrations detected in the neonates, toddlers, and young school age children was significantly lower than levels documented in adults with occupational or environmental exposure. No association of urinary glyphosate was found with any of the three renal biomarkers (KIM-1, NGAL or ACR). Urinary excretion of albumin and serum creatinine concentration are conventional biomarkers for renal dysfunction. Novel biomarkers such as urinary excretion of KIM-1 and NGAL have been developed and it has been that they have diagnostic utility for earlier detection of acute kidney injury (AKI) prior to significant loss of function. KIM-1 also predicted renal damage from acute exposures in animal studies. This study has a number of limitations.

We used maternal education as a proxy for family educational attainment or socioeconomic status (SES) which are well-known factors for different levels of chemical exposures in children or renal injury outcome. We emphasize that low sample size also limited us to perform analyses in the three cohorts in conjunction, in lieu of stratified analysis. We acknowledge that the strength of any conclusion about the frequency of detecting glyphosate in newborns is limited by the sample size. The source of the glyphosate in the newborns, which was detected more frequently than in the other groups but not at high levels, could be via lactation or infant formula. It is possible that immature or different metabolic pathways for glyphosate in neonates, kidney injury during delivery, or lactation may have affected the findings in this age group. However, the addition of neonates in our study evaluating the association of glyphosate and kidney injury biomarkers may have biased findings towards the null. Our results also suggest that glyphosate levels declined with increasing age. The differences by age may represent differences in dietary behavior and access to foods free of contamination or improved elimination of the herbicide, though further research is needed. Previous larger scale case-control studies have shown in the past changes in renal function, kidney injury, or chronic kidney disease of unknown etiology (CKDu) upon glyphosate exposure, especially of adult occupational exposures. Several strengths in this study are notable. Our study is the first to document the prevalence of glyphosate exposures in young children through age 8 years. Our frequent detection of glyphosate suggests the need for inclusion and biomonitoring of glyphosate exposures in national surveys or screenings in populations of interest, which could provide more information in regards to these exposures and outcomes. Glyphosate biomonitoring could be added into studies such as the National Health and Nutrition Examination Survey (NHANES). Our study is novel in that we examine specific renal biomarkers in relationship to glyphosate in a young population. The lack of evident renal toxicity in association with glyphosate exposure in young children does not exclude a potential adverse impact of longer term exposure to the pesticide. Problems during labor and delivery can compromise renal perfusion leading to increased excretion of tubular injury biomarkers in the neonatal period. This could interfere with the ability to detect an independent effect of glyphosate in this age group. In addition, the regenerative capacity of the renal tubular epithelium in infants and young children may mask an adverse effect of the pesticide on tubule integrity. Urinary biomarkers such as NGAL and KIM-1 have been demonstrated to be useful biomarkers of tubular injury even without reduced kidney function, highlighting their utility in detecting subclinical insults to the kidney. Further research could explore more in detail the association between glyphosate and kidney injury biomarker types across different age groups in larger cohorts, and assess whether more prolonged exposure could have progressive deleterious effects.

Conclusion

In this study, we identify frequent prevalence of levels of glyphosate (11.1%), present across all three age ranges, and particularly present in the youngest population of neonates. There is no evidence for renal injury in young children exposed to low levels of glyphosate. Further studies of larger sample size are indicated to better understand the potential deleterious effects of the herbicide after different levels, routes, and duration of exposure.

Assessment and conclusion by applicant:

This study evaluated three cohorts across different phases of child development and measured urinary levels of glyphosate. They evaluated associations of glyphosate with three biomarkers of kidney injury: ACR, NGAL, and KIM-1. Sample collection and analysis as well as statistical evaluation of data have been conducted using well described methodologies. Multivariable regression models failed to identify significant associations of log-transformed glyphosate with any of the kidney injury biomarkers, controlling for covariates age, sex, and maternal education. The authors confirm detectability of glyphosate in children's urine at various ages and stages of life, there is no evidence in this study for renal injury in children exposed to low levels of glyphosate.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good monitoring study.

Reliability criteria of exposure studies from the applicant

Publication: Trasande et al., 2020.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	Y	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	
Exposure to formulations with glyphosate combined with other a.s.	Y	
Exposure to various formulations of pesticides	Y	Environmental exposure.
Study		
Study design clearly described	Y	Longitudinal birth cohort and 2 cross sectional studies. Study of the association between renal biomarkers and glyphosate in urine.
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	Environmental exposure.
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Statistical analysis	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good monitoring study.		

Assessment and conclusion by RMS:

Agreed with the assessment and conclusion by the applicant. The study is considered reliable without restrictions.

This study showed detectability of glyphosate in 8-30% of samples of children's urine obtained from children at

various ages. The mean urinary glyphosate concentration was 0.278 ± 0.228 ng/mL, with a range of 0.105-2.125 ng/mL. There was no evidence for renal injury in children exposed to low levels of glyphosate.

Note RMS: the public literature studies 12 to 29 were provided by the applicant (2 September 2020) upon request by AGG after screening of the results from the literature search. The applicant has submitted the original articles and a short summary, which are provided in the sections below. No assessment on reliability and relevance has been provided by the applicant.

B.6.9.8.12. Literature study 12

Literature study 12 - Acquavella et al. 1999

Data point	KCA 5.9.7
Report author	Acquavella J. F. *et al.*
Report year	1999
Report title	Human ocular effects from self-reported exposures to Roundup® herbicides
Report No	-
Document No	Human & Experimental Toxicology (1999), Vol. 18, pp. 479-486
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	Ocular effects from reported human exposures to Roundup® herbicides were reviewed based on 1513 calls to an American Association of Poison Control Centers (AAPCC) certified regional poison center during the years 1993 through 1997. Relevant records were selected from the AAPCC data base of callers who reported ocular or dermal/ocular exposure to formulated glyphosate herbicides. The AAPCC ranking scheme for a general assessment of severity of effects allows for three levels of adverse effect: minor, moderate and major. Only the latter category concerns effects that are persistent and may not resolve entirely. For the patient records with AAPCC moderate or major outcomes the criteria for the classification and labelling of dangerous substances laid down in the EU Directive were applied additionally. The preponderance of reported exposures were judged by poison center specialists to result in either no injury (21%) or transient minor symptoms (70%). There was some temporary injury in 2% of cases; one injury took more than 2 weeks to resolve. In no instance did exposure result in permanent change to the structure or function of the eye. Since the representativeness of calls to poison control centers is unknown, several interpretations of these findings are possible. The most conservative interpretation is that there were no serious ocular effects from exposure to Roundup® herbicides during a 5 year period among callers to a single regional poison center. A less conservative interpretation would be that severe ocular effects from Roundup® exposures are rare among users of

	these products.
Short description of findings	In summary, medical outcomes for more than 1500 reported human ocular exposures to various Roundup® formulations were reviewed. Most calls involved exposure to dilute formulations. With one possible exception, none of the exposures resulted in persistent effects to the exposed subjects. In no case did exposure result in permanent change to the structure or function of the eye. No clear relationship could be identified between symptoms and exposure to more or less concentrated formulations.
Relevance of this literature article to the submission	The authors evaluated 1513 reported ocular exposures to formulated glyphosate over a 5-year period and found that no permanent injuries were reported to the structure of the eye. They conclude that no serious ocular effects with formulated glyphosate exposures were reported to one regional poison center over 5 years and that based on their data it appears that severe effects are rare.
Comments RMS:	None

B.6.9.8.13. Literature study 13

Literature study 13 - Acquavelle et al. 2004

Data point	KCA 5.9.7
Report author	Acquavella, JF, Alexander, BH., Mandel, JS., Gustin, C., Baker, B., Chapman, P., Bleeke, M.
Report year	2004
Report title	Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study
Report No	-
Document No	Environmental health perspectives (2004), Vol. 112, Issue 3, 321-326; https://doi.org/10.1289/ehp.6667
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	Glyphosate is the active ingredient in Roundup agricultural herbicides and other herbicide formulations that are widely used for agricultural, forestry, and residential weed control. As part of the Farm Family Exposure Study, we evaluated urinary glyphosate concentrations for 48 farmers, their spouses, and their 79 children (4-18 years of age). Farm families were randomly selected from state listings of licensed pesticide applicators in South Carolina and Minnesota. Selection criteria included farmers who lived on the farm and had to farm at least 10 acres within 1 male of the family residence. We evaluated 24-hr composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Urine samples were analysed for glyphosate concentrations by method of chelation ion exchange for the concentration and isolation of glyphosate, followed by quantitation using high-performance liquid chromatography with postcolumn reaction and fluorescence detection. The

	method has a limit of detection (LOD) of 1 µg/L (or 1 ppb) for a 100 mL urine sample. In the calculations a value of 0.5 ppb (LOD/2) was assigned for concentrations that were below the LOD. All the farmers used tractors and boom sprayers, and most applied the Roundup Ultra formulation over glyphosate-tolerant crops early in the growing season. Skin contact with the glyphosate formulation was observed for 31 % of farmers and approximately 5% of farmers were observed to have had spills during mixing and loading or application. 27% repaired their equipment at some time during the application. Sixty percent of farmers had detectable levels of glyphosate in their urine on the day of application. The geometric mean (GM) concentration was 3 ppb, the maximum value was 233 ppb, and the highest estimated systemic dose was 0.004 mg/kg. Farmers who did not use rubber gloves had higher GM urinary concentrations than did other farmers (10 ppb vs. 2.0 ppb). The number of acres treated was not correlated with urinary glyphosate concentration, but there was a trend between concentration and the number of times farmers mixed and loaded the concentrated herbicide formulation. Other factors associated with urinary concentration were using an open cab tractor, observed skin contact with the glyphosate formulation, and repairing equipment during the application. For spouses, 4% had detectable levels in their urine on the day of application. Their maximum value was 3 ppb. For children, 12% had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 ppb. All but one of the children with detectable concentrations had helped with the application or were present during herbicide mixing, loading, or application. The maximum urinary concentration for a child, 29 ppb, was for a teenage boy who actively assisted his father with the mixing and application. The boy's father had the highest urinary concentration among applicators. The field notes documented long periods spent by the farmer repairing the boom sprayer and evidence of spills while mixing and loading. The use of protective gloves was not observed for father or son during mixing or loading or during the repairs. The father was also observed to smoke cigarettes while repairing the boom sprayer. None of the systemic doses estimated in this study approached the U.S. Environmental Protection Agency reference dose for glyphosate of 2 mg/kg/day. The systemic dose was calculated for all farmers, and for all spouses and children who had detectable urinary levels of glyphosate, by calculating the amount of glyphosate excreted during the study period, adjusting for incomplete excretion, adjusting for pharmacokinetic recovery, and dividing the total corrected excretion by each individual's body weight. Nonetheless, it is advisable to minimize exposure to pesticides, and this study did identify specific practices that could be modified to reduce the potential for exposure.
Short description of findings	In only 60% of the participating farmers, urinary glyphosate was detected on the day of handling glyphosate, declining to 27% on the third day after handling glyphosate. The concentration of detected glyphosate in urine was lower in farmers wearing protective equipment (rubber gloves). The size of the field treated with glyphosate was not correlated with urinary concentration but the following factors were associated with urinary concentration: Number of mixing and loading the concentrated herbicide formulation, using an open cab tractor, skin contact, repairing equipment during application. For children, it appeared that most detectable exposures could have been prevented or minimized by avoiding the immediate vicinity during pesticide mixing or application.

Relevance of this literature article to the submission	This study followed farmers who were occupationally exposed to formulated glyphosate and their families and conducted 24 hour urine collections to evaluate glyphosate concentrations before, the day of and for 3 days after an application. They found that there were low urine concentrations in exposed farmers, their children and spouses. The doses were far below the reference dose established by the EPA. The maximum systemic dose in exposed farmers was 0.004 mg/kg. Importantly, 40% of the participant farmers had undetectable urinary concentrations. Urinary concentrations were significantly lower in farmers who wore gloves.
Comments RMS:	None

B.6.9.8.14. Literature study 14

Literature study 14 **Bando et al. 2010**

Data point	KCA 5.9.7
Report author	Bando, H., Murao, Y., Aoyagi, U., Hirakawa, A., Iwase, M., Nakatani, T.
Report year	2010
Report title	An extreme hyperkalemia in a patient with a new glyphosate potassium herbicide poisoning: Report of a case
Report No	-
Document No	Chudoku Kenkyu 23 (3):246-249. (Japanese) Including certification of translation.
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	[Case report] A 65-year-old female was transferred to our emergency and critical care center after taking two kinds of commercially available glyphosate herbicide products. On admission, her conscious level was depressed to Glasgow Coma Scale E3, V2, and M6. Vital signs were as follows; blood pressure 83/33 mmHg, pulse 59/min, and respiratory rate was 24/min. Arterial blood gas analysis showed metabolic acidosis and an extreme hyperkalemia of 9.22 mEq/L. Electrocardiogram showed absence of P wave and a tall, tapering T wave. On admission, gastric lavage was followed by an intragastric administration of activated charcoal together with cathartic. Immediately after recognition of hyperkalemia, sodium bicarbonate, glucose plus insulin, and calcium gluconate were also administered intravenously. Five hours later, plasma concentration of potassium decreased to 4.31 mEq/L, and the patient discharged on day 10. Later, it was disclosed that the new Roundup MaxloadTM contains high concentration of glyphosate potassium. In case of RoundupTM poisoning, we have to take it consideration that the poisoning may results in a hyperkalemia. Typically, the cause of hyperkalemia is believed to be either pseudo-hyperkalemia, potassium leakage from cells, potassium secretion disorder of the kidneys, or excessive potassium intake. Given the frequent blood tests and characteristic electrocardiogram seen in this patient, we were able to rule out pseudo-hyperkalemia. Although we could not rule out the possibility of potassium leakage from cells given metabolic acidosis, that alone would not explain the elevated potassium level of 9.86 mEq/L. As far as the possibility of potassium secretion dysfunction in the kidneys was concerned, this seemed unlikely because BUN was 14 mg/dL, Cr was 0.78 mg/dL, and urinary volume was maintained. However, the patient's hypertension had been treated previously by administration of telmisartan, and the reninangiotensin-aldosterone system had been suppressed by telmisartan, which is an angiotensin II receptor antagonist, and the possibility that this was linked to elevated potassium levels could not be ruled out. Finally, although there was a possibility of excess intake of potassium, the patient said in an interview after her recovery that on that

	particular day she had not taken any other poisons besides the poisonous herbicides containing glycol phosphate (GLYP). When the authors contacted Monsanto, the developer of Roundup, we learned that 100 mL of the product contains 254.4 mEq of potassium. Thus, if this patient had poisoned herself with the full 350 mL of Roundup Maxload®, she would have ingested 890.4 mEq of potassium.
Short description of findings	The article discusses a case of severe hyperkalemia after poisoning with an herbicide containing glycol phosphate (GLYP). In this case, it is believed that the ingestion of Roundup Maxload® containing glyphosate potassium salt was involved in the patients hyperkalemia. When treating GLYP poisoning in the future, the possibility of a potentially lethal condition due to hyperkalemia must be dealt with. Therefore, it is recommended that practitioners check the ingredients of products that have been used in cases of poisoning to ascertain the content of glyphosate potassium salt in order to treat such patients effectively.
Relevance of this literature article to the submission	This is a case report of a formulated glyphosate overdose who developed significant hyperkalemia during her clinical course. This is not unexpected in formulations containing potassium salts. The patient ingested 100 mL of the product containing 254.4 mEq of potassium. The cardiac effects are consistent with hyperkalemia. The patient received standard of care treatment for hyperkalemia and was discharged home.
Comments RMS	None

B.6.9.8.15. Literature study 15

Literature study 15 Barbosa et al. 2001

Data point	KCA 5.9.7
Report author	Barbosa E.R. Elan D. Louis MD, MS, Linda Winfield RN, MPH, Stanley Fahn MD, Blair Ford M
Report year	2001
Report title	Parkinsonism After Glycine-Derivate Exposure Movement Disorders
Report No	-
Document No	Movement Disorders, (2011), Vol. 16, No. 3, pp. 565-568
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	A 54-year-old man accidentally sprayed himself with the chemical agent glyphosate, a herbicide derived from the amino acid glycine. He developed disseminated skin lesions 6 hours after the accident. One month later, he developed a symmetrical parkinsonian syndrome. Two years after the initial exposure to glyphosate, magnetic resonance imaging revealed hyperintense signal in the globus pallidus and substantia nigra, bilaterally, on T2-weighted images. Levodopa / benserazide 500 / 125 mg daily provided satisfactory clinical outcome.
Short description of findings	A Previously healthy 54-year-old man sustained a chemical exposure while spraying glyphosate in a garden. He was wearing no protection gear such as gloves or a face mask, and the exposure to the aforementioned chemical agent occurred as the breeze blew the spay back onto his trunk, arms, legs and face. The substance was not washed off his body until 30 minutes later. He was immediately brought to medical attention and 6 hours after the exposure he developed severe conjuctival hyperemia and a generalized cutaneous rash. One week after the chemical exposure, the skin lesions became blisters that persisted for approximately 15 days. One month after the initial exposure, the patient displayed rigidity and lowness in all four limbs. One year later, he developed a resting slow tremor in the left hand and arm and complained of impaired short-term memory. Family history was unremarkable. In conclusion, it is proposed that the parkinsonian syndrome recorded in this patient might be related to the toxic effects of glyphosate, probably due to an excitotoxic mechanism.

Relevance of this literature article to the submission	This is a case-report describing a patient who developed a Parkinsonian syndrome after being accidentally sprayed with what was said to be a glyphosate based formulation. Shortly after the exposure he developed a diffuse skin lesions which is entirely inconsistent with dermal exposure to glyphosate based herbicides. 30 days later he developed an atypical Parkinsonian syndrome. The authors do not establish that there was an actual glyphosate exposure as they did not obtain a concentration. Additionally, glyphosate is not neurotoxic, and extrapyramidal symptoms have not been reported in cases with large intentional ingestions.
Comments RMS	This study has been assessed previously by the former RMS DE (refer to table below). The current RMS agrees with this assessment.

Reliability of study:	Not assignable
Comment:	Medical case report, single incident
Relevance of study:	Relevant with restrictions (Data are limited due to the absence of any information on purity and application concentrations of glyphosate formulation, as well as co-formulations.)
Klimisch code:	4

B.6.9.8.16. Literature study 16

Literature study 16 Bradberry et al. 2004

Data point	KCA 5.9.7
Report author	Bradberry, SM., Proudfoot, AT., Val, JA.
Report year	2004
Report title	Glyphosate poisoning
Report No	-
Document No	Toxicol Rev. 2004;23(3):159-167. doi:10.2165/00139709-200423030-00003
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	The mechanisms of toxicity of glyphosate formulations are complicated. Not only is glyphosate used as five different salts but commercial formulations of it contain surfactants, which vary in nature and concentration. As a result, human poisoning with this herbicide is not with the active ingredient alone but with complex and variable mixtures. Therefore, it is difficult to separate the toxicity of glyphosate from that of the formulation as a whole or to determine the contribution of surfactants to overall toxicity. Experimental studies suggest that the toxicity of the surfactant, polyoxyethyleneamine (POEA), is greater than the toxicity of glyphosate alone and commercial formulations alone. There is insufficient evidence to conclude that glyphosate preparations containing POEA are more toxic than those containing alternative surfactants. Although surfactants probably contribute to the acute toxicity of glyphosate formulations, the weight of evidence is against surfactants potentiating the toxicity of glyphosate. Accidental ingestion of glyphosate formulations is generally associated with only mild, transient, gastrointestinal features. Most reported cases have followed the deliberate ingestion of the concentrated formulation of Roundup® (41% glyphosate as the IPA salt and 15% POEA). There is a reasonable correlation between the amount ingested and the likelihood of serious systemic sequelae or death. Advancing age is also associated with a less favourable prognosis. Ingestion of >85mL of the concentrated formulation is likely to cause significant toxicity in adults. Gastrointestinal corrosive effects, with mouth, throat and epigastric pain and dysphagia are common. Renal and hepatic impairment are also frequent and usually reflect reduced organ perfusion. Respiratory distress, impaired consciousness, pulmonary oedema, infiltration on chest x-ray, shock, arrythmias, renal failure requiring haemodialysis, metabolic acidosis and hyperkalaemia may supervene in severe cases. Bradycardia and ventricular arrhythmias are often present pre-terminally. Dermal exposure to ready-to-use glyphosate formulations can cause irritation and photo-contact dermatitis has been reported occasionally; these effects are probably due to the preservative Proxel® (benzisothiazolin-3-one). Severe skin burns are very rare. Inhalation is a minor route of exposure but spray mist may cause oral or

	nasal discomfort, an unpleasant taste in the mouth, tingling and throat irritation. Eye exposure may lead to mild conjunctivitis, and superficial corneal injury is possible if irrigation is delayed or inadequate. Management is symptomatic and supportive, and skin decontamination with soap and water after removal of contaminated clothing should be undertaken in cases of dermal exposure.
Short description of findings	The deliberate ingestion of concentrated glyphosate-containing formulations results in severe toxicity and death in some 10–15% of cases, depending on the amount ingested. There is still controversy as to the precise mechanisms of toxicity of the formulations, particularly the role of the surfactant POEA in inducing toxicity. It is unclear also whether non-POEA containing formulations are less (or even more) toxic than POEA-containing formulations.
Relevance of this literature article to the submission	This is a review article discussing the MOA of glyphosate based herbicides, the clinical features of acute overdose and how to manage formulated glyphosate ingestion. From 2001-2003 13,318 glyphosate exposures were reported to the American Association of Poison Control Centers - out of those exposures 5 patients died, demonstrating the very-low toxicity of this product. This review article does not add any new clinical findings to the body of literature.
Comments RMS:	None

B.6.9.8.17. Literature study 17

Literature study 17 **Brunetti et al. 2020**

Data point	KCA 5.9.7
Report author	Brunetti R. Maradey, Joan A.; Dearmin, Rebecca S.; Belford, Peter Matthew; Bhave, Prashant D., MD, FHRS
Report year	2019
Report title	Electrocardiographic abnormalities associated with acute glyphosate toxicity
Report No	-
Document No	HeartRhythm Case Reports, (2019), Vol. 6, Issue 2, pp. 63-66 https://doi.org/10.1016/j.hrcr.2019.10.014
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	A 30-year-old woman with past medical history of asthma presented after a witnessed syncopal episode, without prodrome, at home. Per her family, she was unconscious for at least 30 seconds and spontaneously awoke without requiring cardiopulmonary resuscitation. Family and patient denied any postsyncopal symptoms. The patient denied any history of prior syncopal events. She denied any use of illicit drugs or alcohol. She is a 1-pack-per-day smoker. Family history was positive for stroke in mother. There was no history of sudden cardiac death in her family. Further review of symptoms was positive for a rash on her hands and 10-pound weight gain over 3 months. Home medications consisted of albuterol inhaler and Valium 0.5 mg that the patient reported taking approximately "5 times per month." Upon arrival to the emergency room, the patient's vital signs were as follows: temperature of 99.3°F, heart rate of 78 beats per minute, blood pressure of 79/52, respiratory rate of 20, SpO2 of 94%. The patient was given an intravenous bolus of 2 liters of lactated Ringer's solution, and within 30 minutes her blood pressure had improved (to 130s/70s). Prior to laboratory results and after initial ECG, the patient was treated with 20 mg bicarbonate, 10 units of insulin with dextrose, and calcium gluconate because of presumed hyperkalemia. Comprehensive metabolic panel and complete blood count were both unremarkable, including no electrolyte abnormalities. Brain natriuretic peptide was within normal limits and troponin levels were negative, below the limit of detection for our laboratory. Urine toxicology was negative for all metabolites. Cardiac examination showed regular rate and rhythm without murmur. No S3 or S4 were noted. Jugular venous pressure was <5 cm at 45 degrees and no pedal edema was present. Pulmonary examination was unremarkable. The patient was fully alert and oriented, with no overt neurological deficits on examination. Baseline ECG from 10 years prior showed sinus tachycardia. ECG on admission to this hospitalization, obtained approximately 4 hours post syncope, showed sinus rhythm at 75 beats per minute with first-degree atrioventricular (AV) block (PR 260 ms), LBBB (QRS 200 ms), and significantly prolonged QTC (670 ms) (Figure 1). An ECG done 8 hours

	after syncope showed sinus rhythm, prolonged PR interval, and coved ST segment in leads V1-V2, consistent with a Brugada type 1 pattern. ECG done on hospital day 2 demonstrated sinus rhythm with normal PR interval and narrow QRS). During the admission, the patient did not experience any significant cardiac or respiratory symptoms. In addition to the fluid resuscitation initiated in the emergency department (2 liters), the patient received an additional 2 liters of normal saline during hospital day 1. However, throughout the remainder of the admission, the patient spontaneously diuresed 8.5 liters and was net negative approximately 2.5 liters at time of discharge. No diuretics were administered. In addition to electrolyte imbalances (which were excluded in the emergency room), our differential for her syncope and ECG changes included medication adverse effects, other drug toxicity, or a genetic sodium channel disorder. Throughout the hospitalization, 3 separate rounding teams inquired about suicidal intentions, initiation of new medications, and illicit drug use. The patient consistently denied the above on each occasion. Exposure to herbicides or pesticides was discussed on day 1, which the patient initially denied. On hospital day 2, after mentioning specific brands of pesticides and herbicides, she admitted that she had been using Roundup in her yard. She reported using Roundup super concentrate (50% glyphosate concentrate) the prior evening. She had been using this product regularly while doing yard work for several weeks, without any protective gloves. On several occasions, including the day prior, she had spilled a small amount of the solution on her hands. The patient consistently reported this exposure was accidental, denying any intentional ingestion. She further denied any depressive symptoms, suicidal ideation, or prior history of self-harm. Imaging studies, including chest radiograph and noncontrast computed tomography head and abdomen, were unremarkable. A noncontrast computed tomography chest showed mild pulmonary edema. Telemetry monitoring did not identify any further arrhythmias. Further workup with transthoracic echocardiogram showed normal ejection fraction and paradoxical septal wall motion. Cardiac magnetic resonance imaging was also unremarkable, without evidence of late gadolinium enhancement or myocardial inflammation. She was discharged on hospital day 3 with scheduled follow-up. An ambulatory patch monitor, worn for 14 days, subsequently showed sinus rhythm with narrow QRS and no arrhythmias.
Short description of findings	See above
Relevance of this literature article to the submission	There were no clear causes or exposures other than concentrated glyphosate. This article claims that dermal exposure to a small amount of glyphosate led to a cardiac arrhythmia and claims that the patient developed a Brugada syndrome & long Qt syndrome after exposure. They measured the QTC in a wide-complex tracing which is uninterpretable. Brugada syndrome is largely due to sodium channel blockade in the cardiac myocytes, LQT syndrome is largely due to potassium channel blockade in the cardiac myocytes. Glyphosate does neither. Moreover, glyphosate is not dermally absorbed and multiple GLP studies have shown that glyphosate is not cardiotoxic
Comments RMS:	Agree that no cardiotoxic effects are reported upon ingestion of small amounts. Considering the dermal absorption, it is disagreed that glyphosate is not dermally absorbed, however, the dermal absorption is low. As no further information on the actual exposure of the patient is provided it is not possible to conclude on causality.

B.6.9.8.18. Literature study 18

Literature study 18 Chang et al. 1999

1. Information on the literature article

Data point	KCA 5.9.7
Report author	CY Chang, YC Peng, DZ Hung, WH Hu, DY Yang, TJ Lin
Report year	1999
Report title	Clinical impact of upper gastrointestinal tract injuries in glyphosate-surfactant oral intoxication
Report No	-
Document No	Human & Experimental Toxicology, (1999) Vol. 18, pp. 475-478
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	This publication reports of the cases of glyphosate-surfactant herbicide poisoning seen in the emergency department of the Veterans General Hospital in Taipei, Taiwan collected from January 1994 through June 1998. Glyphosate-surfactant herbicide poisoning was diagnosed by an accompanying container, or recognized by the patients themselves or by their families. Patients who ingested glyphosate-surfactant accompanied by other poisons were excluded. The amounts ingested were obtained from the chart records. If the ingested amount was recorded with descriptive terms, it was estimated with the following rules: ‘a little’ or ‘a spoon’ as 5 ml, ‘a mouthful’ as 25 ml, ‘a small cup’ as 100 ml, and ‘a bottle’ as 300 ml. Fifty patients with glyphosate-surfactant oral ingestion were studied with upper gastrointestinal (UGI) endoscopic grading using Zargar's modified grading system for mucosal corrosive injury within 48 hours after oral intoxication. Esophageal injury was seen in 68% of the patients, gastric injury in 72%, and duodenal injury in 16%. There were no grade 3 injuries. The upper gastrointestinal tract injuries caused by glyphosate-surfactant were minor in comparison with those by other strong acids. The WBC count, amount of glyphosate-surfactant ingested, length of hospital stay and the occurrence of serious complications increased markedly in the group which had grade 2 esophageal injuries. Thus, the severity of the esophageal injuries may be a prognostic factor for the patient with glyphosate surfactant ingestion. The UGI endoscopy may be indicated for grading esophageal injury in patients who have ingested glyphosate-surfactant in amounts greater than 100 ml. Physicians should pay more attention to the patients with grade 2 or 3 esophageal injuries to prevent serious complications and to provide aggressive supportive care.
Short description of findings	See above.

Relevance of this literature article to the submission	In acute large volume ingestions of formulated glyphosate it is common to see corrosive injury to the gastrointestinal tract. This is a case series of fifty patients who had upper GI studies after ingesting formulated glyphosate. Since the esophagus does not have a serosa, it is harder to repair after a corrosive injury, so it is not surprising that esophageal injury has prognostic.
Comments RMS:	None

B.6.9.8.19. Literature study 19

Literature study 19 Goldstein et al. 1999

Data point	KCA 5.9.7
Report author	Daniel A. Goldstein, MD Glenn Johnson, CIH Donna R. Farmer, PhD Mark A. Martens, PhD Rafael S. Carel MD, DrPH, Luba A. Pushnoy, MD
Report year	1999
Report title	Pneumonitis and Herbicide Exposure
Report No	-
Document No	CHEST, (1999), Vol. 116, No. 4, pp. 1139
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	The authors (Goldstein *et al.*) agree with Pushnoy et al. that acute-care physicians frequently assume that harmful effects resulting from exposures to agricultural herbicides are due to organophosphate compounds. However, the authors disagree with the authors' conclusion that the pneumonitis in this case was the result of exposure to Roundup (Monsanto Co; St. Louis, MO). No information was provided to demonstrate how airborne exposure could have occurred. Neither glyphosate (an organophosphonate herbicide that does not inhibit cholinesterase) nor any components of the finished product (Roundup) become significantly airborne through vaporization, even in a poorly ventilated space. While the equipment being repaired could have generated an aerosol, such equipment would normally contain a highly diluted form of the product and would produce a droplet of non-respirable size (200+ mm). Skin absorption of Roundup is inconsequential and has never been demonstrated to cause pulmonary injury. In short, there is no plausible route of exposure to Roundup in this case, making a causal relationship highly unlikely. Occupational pneumonitis has never been reported in connection with Roundup. Although there are reports of the aspiration of Roundup concentrate resulting in lung injury (Tominack et al., and Talbot et al.) almost all reported pulmonary effects have occurred following suicidal ingestion of the undiluted Roundup concentrate and appear to be the result of cardiovascular toxicity producing non-vasogenic pulmonary edema (Tominack et al., and Talbot et al.). Further, clinical experience with ingestion or aspiration is not applicable to purported vapor inhalation. Other possible sources of exposure not discussed by Pushnoy et al. include the following: use of compressed air (generating an aerosol consisting predominantly of diesel fuel); use of a chlorinated solvent in conjunction with smoking (generating phosgene); welding (metal fume fever); or the occurrence of unreported pulmonary aspiration (siphoning or blowing out parts using the mouth). Non-occupational causes consistent with the clinical course have not been excluded. Physicians are often quick to conclude that a reported chemical exposure is

responsible for a temporally associated clinical illness. In such cases, a thorough medical history, a situation-specific exposure assessment, and an evaluation of the physical and toxicologic properties of the material in question are essential if valid conclusions are to be drawn. Further investigation of this case may have revealed a more plausible occupational cause.
Lacking this, the relationship between occupational activity and pulmonary pathology in this case is more likely coincidental.

Carel & Pushnoy:
The points brought up in the letter of Goldstein *et al.* are well taken, and some of them were not addressed in our article (December 1998) due to the concise presentation of the case report. However, as we have taken a detailed occupational history from the concerned patient, we can reject most of the alternative exposure suggested by Goldstein *et al.* (welding, use of compressed air, use of chlorinated solvents, or nonoccupational causes). It is also known that dermal absorption is very low. As we stated in our article, the patient worked in a small, hot room with a ventilator blowing over the bucket containing the clogged parts and manifolds toward him. He worked on cleaning the parts continuously for 4h. Even though the vapor pressure of glyphosate is relatively low at 25°C, we have concluded that the patient's clinical symptomatology resulted from exposure and inhalation of a mixture of vapor and air-borne droplets containing glyphosate. This conclusion is based mainly on two facts: (1) the clinical picture of burns of the mucosal lining of the pharynx and larynx (glyphosate is a weak organic acid, highly soluble in water); and (2) the pulmonary parenchymal picture of acute toxic pneumonitis. Technical glyphosate contains an active ingredient (N-phosphonomethyl-glycine) plus surfactants and inert substances. It is our assumption that part of the parenchymal reaction was due to the effect of a surfactant (such as polyoxyethylene amine) on the alveolar lining, leading to extensive alveolar involvement, as was shown on the chest radiograph. This effect of an external surfactant on lunch parenchyma seems quite plausible, but admittedly, based just on clinical evidence. In conclusion, we maintain that this worker was affected by exposure to vapors and air-borne particles of Roundup and that he developed an acute pulmonary reaction to this exposure.

Short description of findings Not applicable, letters to the editor.

Relevance of this literature article to the submission	Letter to the editor commenting on the article: Pushnoy L. A. et al., Herbicide (Roundup) pneumonitis. Chest, (1998); Vol. 114, pp. 1769–1771). Refer to public literature study 24. This is a letter to the editor disputing a claim that formulated glyphosate exposure led to pneumonitis. Formulated glyphosate does not vaporize, nor are the droplets produced in a sprayer of respirable size so the claim that occupational pneumonitis is related to this type of exposure is not plausible. This letter provides further arguments on the non-relevance of Pushnoy article to the submission. Further references cited above: Tominack R. L. et al. Taiwan National Poison Center survey of glyphosate-surfactant herbicide ingestions. Clin. Toxicol., (1991), Vol. 29, pp. 91-109. Talbot A. R. et al. Intentional self-poisoning with glyphosate containing herbicides (Roundup): a review of 93 cases. Hum. Exp. Toxicol., (1991), Vol. 10, pp. 1-8 This letter is answered by a letter from Pushnoy and Carel addressing the arguments made by Goldstein *et al.* The authors reject most of the alternative exposures suggested by Goldstein *et al.* and maintain the conclusion that the worker was affected by exposure to vapors and air-born particles of the solution containing glyphosate based on the clinical evidence.
Comments RMS:	None

B.6.9.8.20. Literature study 20

Literature study 20 Goldstein et al. 2002

Data point	KCA 5.9.7
Report author	Daniel A. Goldstein, John F. Acquavella, Rhonda M. Mannion & Donna R. Farmer
Report year	2002
Report title	An analysis of glyphosate data from the California Environmental Protection Agency Pesticide Illness Surveillance Program
Report No	-
Document No	Journal of Toxicology. Clinical Toxicology 40(7):885-892 (2013) DOI: 10.1081/clt-120016960
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	Goldstein et al. analyzed glyphosate-related calls to the Pesticide Illness Surveillance Program (PISP) to assess the number of reports involving systemic symptoms and to better understand the nature and severity of reported cases. Data on glyphosate and other pesticides are available for the years 1982-1997 including: type of exposure (agricultural/other); target organ(s) affected (skin/eye/respiratory/systemic); exposure(s); an assessment of causal relationship (possible, probable, or definite); and limited medical text. All individual cases were reviewed by the authors to verify that classifications were appropriate based upon the recorded information and to assure that systemic illness potentially related to glyphosate products was not inadvertently excluded from the analysis.
Short description of findings	A total of 815 callers mentioned glyphosate from 1982 to 1997. Of 815 total glyphosate calls, 464 (57%) were characterized as agricultural and 351 (43%) were characterized as nonagricultural. Most calls involved topical irritation of the eye (n = 399), skin (n = 250), upper airway (n = 7), or combinations of these sites (n = 32) without systemic symptoms. Of the 187 systemic cases, only 22 (<3%) had symptoms recorded as probably or definitely related to glyphosate exposure alone. These symptoms were reported predominantly following dermal exposure, with some limited possibility for concurrent ingestion of small quantities of material. The reported symptoms were not severe, expected to be limited in duration, and frequently inconsistent with the route of exposure and/or previous experience with glyphosate. The authors concluded that call volume is not a reliable indicator of the actual incidence or severity of glyphosate-related incidents in California.

Relevance of this literature article to the submission	This study was conducted in order to assess the number of reports involving systemic symptoms related to glyphosate-based products, and to better understand the nature and severity of these cases reported to California's PISP. From 1982-1997, a total of 815 callers mentioned glyphosate. Out of these only 22 systemic cases were characterized as definitely or probably related to glyphosate exposure alone and only one fatality was reported - a suicide involving a mixed organophosphate and glyphosate ingestion. The article then goes on to describe the limitations of the PISP reporting system.
Comments RMS:	None

B.6.9.8.21. Literature study 21

Literature study 21 **Kamijo et al. 2012**

Data point	KCA 5.9.7
Report author	Kamijo, Y., Mekari, M., Yoshimura, K., Kan'o, T., Soma, K.
Report year	2012
Report title	Glyphosate-surfactant herbicide products containing glyphosate potassium salt can cause fatal hyperkalemia if ingested in massive amounts
Report No	-
Document No	Clinical Toxicology 50:159 (2012). DOI: 10.3109/15563650.2011.648747.
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	This is a letter to the editor reporting a clinical case of refractory ventricular arrhythmia caused by extreme hyperkalemia shortly after the ingestion of a glyphosate-surfactant (GlySH) product.
Short description of findings	A 69-year-old woman ingested about 500 mL of a GlySH product (Roundup Maxload 1L, Nissan Chemical Industries Ltd., Tokyo, Japan). On admission, she lost consciousness and had no pulse. Electrocardiogram (ECG) revealed ventricular tachycardia (VT); the patient received cardiopulmonary resuscitation with endotracheal intubation and cardioversions. VT was refractory and responses to the treatments were only temporary. Laboratory tests revealed extreme hyperkalemia (10.7 mEq/L) with normal renal function (BUN: 17.9 mg/dL, Cr: 0.51 mg/dL) and metabolic acidosis (pH: 7.005, $PaCO_2$: 41.6 mmHg, BE: 20.7 mmol/L, HCO_3: 10.1 mmol/L). The patient was diagnosed with acute respiratory distress syndrome (ARDS). After percutaneous cardiopulmonary support and continuous hemodialysis, the potassium serum concentration promptly decreased to normal levels and ECG showed normal sinus rhythm. Endoscopy revealed pharyngeal edema, as well as esophageal and gastric erosions. Both the ARDS and edema gradually improved. Serum glyphosate levels on admission and after 18 hours were 1625.74 and 100.44 µg/mL, respectively. The authors concluded that, considering the normal renal function and ECG findings, the most probable cause of her hyperkalemia was an excessive intake of potassium. The GlySH product contained about 2.6 mEq/mL of potassium, and the patient took as much as 1300 mEq of potassium. The authors also stated that it may be problematic that GlySH products containing much potassium can be easily purchased in retail stores throughout Japan. They indicated that physicians should be aware that GlySH products containing glyphosate potassium salt can cause fatal hyperkalemia if ingested in massive amounts.

Relevance of this literature article to the submission	This is a letter to the editor reporting a case of hyperkalemia of 10.7 mEq/L related to formulated glyphosate suicidal ingestion. The description of the dysrhythmias and ECG findings are consistent with hyperkalemia and the patient's clinical course improved with intensive care and hemodialysis. The clinical features of this case report are not unusual for suicidal ingestion of the potassium salt formulation.
Comments RMS:	None

B.6.9.8.22. Literature study 22

Literature study 22 **Khot et al. 2018**

Data point	KCA 5.9.7
Report author	Khot, R., Bhise, A., Joshi, R., Ambade, N. P.
Report year	2018
Report title	Glyphosate poisoning with acute fulminant hepatic failure
Report No	-
Document No	Asia Pacific Journal of Medical Toxicology 7(3):86-88 (2018) DOI: 10.22038/apjmt.2018.11984
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	This is a clinical case report of acute fulminant liver failure after the accidental consumption of glyphosate-containing herbicide. The patient's liver function deteriorated despite supportive treatment. Later, he developed hepatorenal syndrome and died. Based on previous studies on rats, the authors discussed the association between chronic exposure to low levels of glyphosate-based herbicides (GlySH) and alterations of the liver proteome and metabolome, and possible development of non-alcoholic fatty liver disease (NAFLD).
Short description of findings	A 20-year-old male patient was referred to the hospital after 5 days of treatment for alcohol drinking with an increased risk of alcohol accidental ingestion of a GlySH herbicide. He had ingested about 25 ml of the liquid when trying to open the lid of the bottle, by holding it between his teeth. He was routinely spraying it on his farm for the last 5 years and used to consume the sprayed grains from his farm. On hospital admission, the patient had stable vital signs, stomatitis, glossitis, multiple mucosal ulcers, and icterus. Laboratory tests revealed normal hemogram and complete blood counts, elevated serum bilirubin, marginally elevated alkaline phosphatase, serum creatinine of 8.6 mg/dL, and blood urea of 179 mg/dL. The patient showed an initial improvement with supportive treatment. However, in the following days, the patient developed mucosal bleeding, increase in ascites, bilateral pleural effusion, gastrointestinal (GI) bleeding, and hepatic encephalopathy. The patient's liver and renal functions continued to deteriorate until he became comatose, developed a massive bout of GI bleed, and died despite all supportive and resuscitative measures. Focusing on the potential unfavorable hepatic impacts of glyphosate, the authors mention two reports on rats by Mesnage et al. In one of the reports, chronic consumption of ultra-low levels of a GlySH formulation (at admissible glyphosate-equivalent concentrations) was associated with marked alterations of the liver proteome and metabolome (Mesnage et al. 2017a). In a second report, Mesnage et al performed an integrated analysis of these molecular profiles, which the authors mention as clearly reflective of features of NAFLD and its progression to non-alcoholic steatohepatitis

(Mesnage et al. 2017b). The authors stated that the patient also had a low-dose long-term exposure to GlySH and might have had underlying NAFLD, suggesting that acute decompensation of the liver function must have occurred following accidental ingestion of GlySH. The authors concluded that chronic, as well as acute exposure to GlySH, could lead to NAFLD and fulminant liver failure.

Relevance of this literature article to the submission

This is a case report of a 20-year-old patient who developed jaundice, ascites, and GI bleeding due to fulminant hepatic failure. The claim is that, in addition to a low exposure from use of glyphosate formulation for 5 year, he had an accidental exposure to 25 mL because he got a small amount in his mouth while opening a bottle with his teeth. He was tested for infectious hepatitis with negative results so the authors concluded that he could possibly have had underlying liver disease and his 25 mL exposure triggered fulminant hepatitis. This report is speculative and relies on invalidated data to support its claims. Although suicidal overdoses may cause liver injury due to corrosive injury of the GI tract, occupational exposures are not associated with the development of liver disease. GLP studies show that glyphosate-based herbicides are not hepatotoxic. The references to the Mesnage paper claiming that there are hepatotoxic effects in the text can be addressed as follows: Mesnage et al. use omics to further analyse the same samples obtained from a 2012 Seralini study that was determined to be not scientifically sound by regulatory authorities [European Food Safety Authority (EFSA) and the German Federal Institute for Risk Assessment (BfR)]. This was due to significant flaws in the study design and interpretation of the data, and it was formally retracted by Food and Chemical Toxicology. Even though it was subsequently republished in 2014, it was done so without peer review (Casassus, B. 2014).

The use of omics is an effort to explain their unsubstantiated conclusion that glyphosate is a cause of liver disease, stating that "... molecular profiling methods can potentially provide [insights] into the processes and mechanisms of toxicity ..." including signs of liver toxicity. However, claimed clinical effects in the Seralini et al. study were widely refuted including within the EFSA analysis, which "… highlighted the lack of any dose-response relationship for the parameters reported" as well as the "lack of a balanced scientific discussion." EFSA also questioned "… the authors' interpretation of biochemical parameters as indicators of kidney and liver failure."

Owing to the fact that liver toxicity was not established in the 2012 study, the premise for doing this current omics analysis is questionable. This is made even more so as omics assays, on their own, are not intended for reaching conclusions pertaining to safety. Rather, omics is a collection of techniques used to generate data for comparison between samples, and even if performed properly, is not sufficient justification, in the absence of corroborating evidence, for acceptance of any conclusion regarding safety or for extrapolating to any clinical condition. The ability of omics assays to be used for diagnostic assessment has not yet been determined; therefore, the results of this study should be carefully considered before reaching a conclusion that there is a safety concern. This is especially true since standardized, validated diagnostic tests are available for kidney and liver toxicity, but these were not used in this study.

Mesnage et al. support their conclusion of toxicity by stating that "… a

number of toxicity studies have shown that glyphosate and its commercial formulations have non-target effects on mammalian metabolism and provoke toxic effects, especially with respect to liver and kidney ..." However, the authors cite two reviews of the literature that they themselves performed as supporting evidence (Mesnage et al., 2015; Myers et al., 2016). These reviews discuss only a subset of the literature, including many studies which have been refuted and/or do not support this statement.

Regarding study design, the liver samples used for this study are from the Seralini et al., 2012 study which was retracted for numerous reasons, including poor study design. Upon further review, this study design is also unsuitable for the current omics analyses.

1. For instance, the control and glyphosate formulation treated animals were euthanized at 701 ± 62 and 635 ± 131 days, respectively. The fact that rats where euthanized on different days and samples harvested on different days is alone sufficient reason to explain any variability observed in the omic assays. If done correctly, samples marked as direct comparators should be collected at the same time and handled in the same way.

2. Rats were administered glyphosate formulations following addition to their drinking water; however, actual exposure can not be calculated as water intakes are not reported. This represents a very critical omission of information because by not measuring water intake it is impossible to know how much glyphosate formulation was ingested.

3. The tissues used for the omics assays are highly confounded by day of age and clinical condition. For example, control and treated rats developed tumors which were allowed to grow. While certainly an ethical concern, this can also create considerable variability that is not accounted for. Additionally, the liver samples were harvested from rats that were moribund or at the end of their lifetime which can impact food consumption. Specifically, liver samples were taken (and frozen) when rats were euthanized due to illness, or at 24 months (end of study). Both the proteomic and metabolomic measurements were made after 24 months. No adjustments were made for time of death, and no data were reported on reason for death or health condition at time of death. It is reasonable to conclude that any of these factors could impact the results of the omics assays.

4. The facts that the fold differences for many of the reported endpoints are quite small (less than 2 fold) in conjunction with the relatively small sample size diminish the authors' ability to properly distinguish "signal" from "noise".

There are also numerous concerns regarding the authors' presentation and statistical analysis of the data as well. Mesnage et al. leave out information vital to reaching the conclusions they put forth.

1. For example, the authors state that "blood was taken via the tail vein of each animal after 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 months of treatment" and that statistical analysis (Mann-Whitney) were computed for blood triglycerides at each date (see Fig. 1). However, the analysis of data for two

dates (months 2 and 24) were not reported. The reader was not informed, nor was a reason provided. This is highly unusual, especially for month 24, the last month of measurements for the study. To be left unreported raises the question as to what were the results at 24 months?

2. The authors performed multiple test corrections resulting in only 3 metabolites showing any difference. The authors then consider the 55 metabolites which were not significantly different (p-value <0.05). This is an obvious effort to support their “biological Interpretation" of the data and should be viewed with scepticism.

3. Another example of crucial information being withheld is the number of dropouts due to mortality, which means some data are missing. Missing data can lead to bias and the authors’ observations could be a result of mortality and not treatments.

4. Another approach used for looking at the data was principal component analysis (PCA) plots which were conducted on both the proteomics and metabolomics data (see Fig. 2A). The data show that there are obvious outliers in the PCA plots that the authors fail to discuss. Notably, the apparent effect of the glyphosate formulation was much smaller than the outlier effect in the Control group in the proteome plot. The absence of discussion regarding the cause of the large outlier effect raises serious concerns regarding how the authors’ conclusions are influenced by these outliers.

5. Benjamini-Hochberg (BH) adjustment was used to counter the effect of inflated errors due to multiple comparisons and multiple testing situations. It is appropriate to do this type of adjustment to address the inflated error rates, but note that on page 9 the authors disregard their BH adjustment. Thus some of the effects listed are almost sure to be false positives. The biological meaning of differences (including the apparent difference in cholesterol levels) should not even be discussed unless the difference is statistically significant.

In summary, the authors’ conclusions are not supported by the data. The study suffers from small sample size, a high level of variability because of numerous confounding factors, and an over-interpretation of the data, including ignoring their own statistical findings in an effort to establish biological relevance when there is none.

References

Mesnage, R., Arno, M. Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. Environ Health 2017;16:28.

Mesnage, R., Renney, G., Séralini, G. E., Ward, M., Antoniou, M. N. Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide. Sci Rep 2017;7:39328.

Myers, J. P. et al. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Environ Health

	2016; 15:19.
RMS comments:	The RMS considers that based on other case reports of relatively small ingestions showing no liver damage and based on the fact that no acute effects are observed on the liver in the guideline animal studies, it is not likely that a small ingestion would cause fulminant hepatic failure.

B.6.9.8.23. Literature study 23

Literature study 23 Lee et al. 2000

Data point	KCA 5.9.7
Report uthor	Lee, H. L., Chen, K. W., Chi, C. H., Huang, J. J., Tsai, L. M.
Report year	2000
Report title	Clinical presentations and prognostic factors of a glyphosate-surfactant herbicide intoxication: a review of 131 cases
Report No	-
Document No	Academic Emergency Medicine 7(8):906-10 (2000) DOI: 10.1111/j.1553-2712.2000.tb02069.x
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	This is a report of a retrospective study of 131 patients treated for intoxication with glyphosate-surfactant herbicide (GlySH) who were admitted to the emergency department of the National Cheng Kung University Hospital from 1988 to 1995. Suicide attempts with agricultural chemicals are common in southern Taiwan. Among them, GlySH intoxication is increasingly frequent. Although the clinical course and outcomes following ingestion have been described, predictors of serious complications and mortality have not been elucidated. Therefore, the objective of this study was to identify the risk factors and prognostic factors in GlySH intoxication. The authors reviewed medical charts and abstracted clinical and laboratory variables, looking for predictors of mortality using univariate analysis and multiple logistic regression.
Short description of findings	In this study, there were 131 patients with GlySH ingestion, 69 of whom were male and 62 female. Nausea with or without vomiting (73.8%), sore throat (79.5%), and fever (41.2%) were the most common initial manifestations. Leukocytosis (68.0%), low bicarbonate (48.1%), acidosis (35.8%), hepatic dysfunction (33.6%), hypercapnia (30.9%), hypoxemia (28.4%), and renal insufficiency (17.1%) were the most common laboratory abnormalities. There were 11 fatalities, for a mortality rate of 8.4%. The mean ± SEM age of the survivors was 47 ± 2 years, while that of those who died was 60 ± 4 ($p = 0.02$). The estimated amount of GlySH ingested averaged 122 ± 12 mL among the survivors and 330 ± 42 mL among those who died ($p < 0.001$). Of the 17 identified variables, eight were highly associated with poor outcome and mortality, including respiratory distress necessitating intubation, respiratory distress, renal dysfunction necessitating hemodialysis, abnormal CXR, shock, larger amount of ingestion (>200 mL), altered consciousness, hyperkalemia, and pulmonary edema. Using multiple logistic regression analysis, the authors identified three factors that could help to predict outcome more precisely: pulmonary edema, acidosis, and hyperkalemia.

	The authors recommended that all the patients who are reported to have ingested large amounts of GlySH be carefully observed, especially those who present in respiratory distress.
Relevance of this literature article to the submission	This is a retrospective review of glyphosate base herbicide overdoses treated at a Tawainese medical center from 1988-1995. The authors aim was to identify prognostic features for severe overdoses. Overall, 131 patients presented to the hospital with GlySH ingestion, 69 of whom were male and 62 female. There were 11 fatalities, for a mortality rate of 8.4%. The authors then identified 3 clinical features that were associated with a significantly increased risk of mortality, including acidosis, hyperkalemia, and pulmonary edema. This paper describes the typical clinical features of severe formulated glyphosate overdoses.
Comments RMS	No comments

B.6.9.8.24. Literature study 24

Literature study 24 **Pushnoy et al. 1998**

Data point	KCA 5.9.7
Report author	Pushnoy L. A. et al.
Report year	1998
Report title	Herbicide (Roundup) Pneumonitis
Report No	-
Document No	CHEST 1998; 114:1769-1771
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	A case of acute intoxication presented as toxic pneumonitis after exposure to Roundup (glyphosate) (Solaris Group, Monsanto; San Ramon, CA) herbicide in an agriculture worker. The correct etiologic factor causing this specific clinical picture was identified only 2 weeks later, after a thorough occupational history was taken and meticulous delineation of the working conditions and exposures of the involved worker were made. As a rule, occupational related diseases are not readily elucidated by non-occupational physicians. However, most acute intoxication events are first encountered by such physicians. In these situations, rapid and comprehensive evaluation is necessary in order to clearly identify the causative agent(s) and to initiate the appropriate treatment. Consulting occupational physicians at this early stage may facilitate early and accurate diagnosis.
Short description of findings	A 42-year-old mechanic entered an ED complaining of shortness of breath, irritative cough, dizziness, discomfort in the throat, and episodes of hemoptysis. Past history revealed no significant health problems, except smoking 20 cigarettes daily for many years. Because of these symptoms, the subject was admitted to the hospital. Earlier that day, the patient had been working in a confined space cleaning and repairing a spraying device mounted on a tractor. He disassembled the clogged sprayers and manifolds from the device and used diesel fuel as a cleaning solvent. The work was performed in a small room with one window. To improve his working conditions, he put a ventilator at one end of the room, thus, moving air over the bucket where the clogged parts were immersed in the solvent. The disassembled parts contained remnants of the spraying solution used. The patient was conscious on admission to the hospital. His temperature was 38.4°C; respiration rate was 30 breaths/min; pulse was regular, 109/min; and BP 116/70 mm Hg. Pulse oximeter showed saturation of 90%. He was in mild to moderate respiratory distress; diffuse rales and crackles were heard over the lungs. The rest of the physical examination results were normal. ECC was normal, and the chest radiograph showed diffuse bilateral alveolar pattern. Laboratory workup showed WBC count 17,700/mm³ with 90% neutrophils. There was no anemia, electrolyte imbalance, or disturbed kidney functions. Liver function test results were normal; pseudocholinesterase level was normal. The patient was treated with IV antibiotics (Augmentin), corticosteroids,

	and oxygen. Within 48 h, he showed marked clinical improvement, and the repeat chest radiograph showed a significant clearing of the pulmonary findings. The patient was discharged with recommendation to continue tapering the prednisolone and antibiotic treatment. Two days later, he returned to the ED complaining of tightness in the chest, hoarseness, and epigastric pain. He was not in any respiratory distress. Otolaryngologic evaluation demonstrated signs of burns in the mucosal membranes of the pharynx and larynx. Chest radiograph showed further improvement compared to the previous one; and 4 days later, the radiograph findings completely cleared. Pulmonary function tests done at that time revealed evidence of moderate restriction: FVC of3.07 L (51% of predicted), FEV 1 of2.66 L (.56% of predicted), and FEV 1/FVC of 86%. All blood test results were within normal range, except impaired liver function (alanine arninotransferase-165 IU), returning to normal only 3 months later.
Relevance of this literature article to the submission	This is a case report of a heavy smoker who developed respiratory symptoms, fever, cough and hemopytsis that improved with steroids and antibiotics. He had been cleaning out his sprayer in a confined space using diesel fuel as a solvent to unclog sprayer parts prior to developing symptoms. The authors claim that his exposure to RoundUp was the likely etiology for his pneumonitis (although there is discussion of the potential involvement of surfactants used in RoundUp formulations). The authors discounted exposure to diesel fumes as a likely cause due to the clinical signs unlikely to develop after exposure to organophosphates or diesel fumes. Occupational exposure to RoundUp formulations have not previously been associated with the development of pneumonitis.
Comments RMS:	No comments.

B.6.9.8.25. Literature study 25

Literature study 25 Sawada et al. 1987

1. Information on the literature article

Data point	KCA 5.9.7
Report author	Sawada Y. and Nagai Y.
Report year	1987
Report title	Roundup poisoning --- its clinical observation possible involvement of surfactant
Report No	-
Document No	Journal of Clinical and Experimental Medicine (IGAKU NO AYUMI) - Vol. 143 No.l p. 25-27 (1987.10.3)
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	This report summarizes 56 poisoning cases of a new herbicide in Japan, Roundup® and clarifies the clinical status of poisoning as well as presents treatment methods. It also suggests that the poisoning may have been caused by the surfactant of Roundup® on the basis of first-hand experience of treating Roundup® poisoning cases.

Short description of findings	Based on the analysis, the authors summarise and understand the pathophysiology of Roundup® poisoning as below:
	- Disorders of Central Nervous System, kidney functions and cardiac conduction, hemolysis
	  o Aspiration pneumoniaHypovolemic shock
	- Irritation and corrosion of digestive tract
	  o Emesis and diarrhea
	  o Bleeding of digestive tract
	  o Intestinal edema
	  o Parlaytic ileus
	  o Hypovolemic shock
	- Promotion of blood vascular permeability
	  o Lowering cardiac function, lowering SRV
	  o Pulmonary edema
	  o anasarca

Relevance of this literature article to the submission	This case series describes the clinical presentation of 56 patients with formulated glyphosate ingestions between 1984-1986. 48 of the cases were attempted suicides, 3 were infant ingestions, presumed accidental and 5 were of unknown circumstance. Nine of the patients died. The reported average dose for those dying was 206 mL versus 104 mL in survivors. The authors describe the clinical course that the patients had and state that it is similar to 2 surfactant ingestions that they cared for in the past (shampoo and spreader) and postulate that the surfactant moiety is responsible for most of the clinical features of the overdose. This is a known feature of formulated glyphosate overdose.
RMS comments	None

B.6.9.8.26. Literature study 26

Literature study 26 Sawada et al. 1988

Data point	KCA 5.9.7
Report author	Yusuke Sawada, Yoshikazu Nagai, Masashi Ueyama, Isotoshi Yamamoto
Report year	1988
Report title	Probable toxicity of surface-active agent in commercial herbicide containing glyphosate.
Report No	-
Document No	The Lancet 1988, p.299
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	No abstract - letter to the editor Note – this letter appears to be a comment on the same data set as reported in Journal of Clinical and Experimental Medicine (IGAKU NO AYUMI) - Vol. 143 No.l p. 25-27 (1987.10.3) by the same author. This summary should be linked to that report where additional information can be found.
Short description of findings	The main clinical findings were: - Gastrointestinal: Sore throat, abdominal pain, and vomiting were noted in almost all cases with haemorrhage and paralytic ileus in serious cases. Endoscopy (in 7 cases) revealed erosion of the pharynx, oesophagus, and stomach. Some cases with no symptoms when examined in hospital later had haematemesis and melaena that lasted several days. Necropsy of those who died within 48 h of ingestion revealed erosion, necrosis, and haemorrhage of the jejunum and ileum, and a large area of oedematous mucous membrane extending from the ileocaecum to the large intestine. - Respiratory: Pulmonary oedema (3 cases) and severe pneumonia (2). - Cardiovascular: Oliguria, anuria, and hypotension in all fatal cases, and transiently in the survivors too. Maintenance of blood pressure and urine output required massive infusions of fluids. - Central nervous system: Moderate clouding of consciousness in some cases. - Laboratory findings: Serum amylase and white cell count were increased; 6/17 cases tested had a serum total bilirubin above 0-9 mg/dl and raised lactate dehydrogenase activity. Haemolysis probably explained these abnormalities. Serum electrolytes were normal.
Relevance of this literature article to the submission	This is a letter to the editor describing the clinical features of formulated glyphosate overdose and postulating that the findings are due to the surfactant in the product. Surfactants cause corrosive injury to the GI tract when ingested so the clinical findings are not unexpected.
RMS comments:	No comments

B.6.9.8.27. Literature study 27

Literature study 27 Tominack et al. 1989

Data point	KCA 5.9.7
Report author	Rebecca Tominack, M.D.,Patrick Conner,hl.D.' NIamoru Yamashlta, kI.D
Report year	1989
Report title	Clinical Management of ROUNDUP Herbicide Exposure
Report No	-
Document No	2 : 187 – 192. 1989
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	Roundup herbicide concentrate, is a formulated product of the isopropylamine salt of glyphosate, surfactant, and water. Accidental exposures to Roundup herbicide have not produced serious toxic effects. It is occasionally deliberately ingested to attempt suicide in Asia. Intentional ingestion of large amounts places the patient at risk for a toxic syndrome involving manifestations of gastrointestinal irritation, cardiovascular compromise, pulmonary congestion and renal dysfunction. There is no specific antidote; the product does not inhibit cholinesterase. Aggressive supportive management is the mainstay of treatment. Hemodialysis and hemoperfusion are unproven but potentially beneficial treatment options. Research into pathophysiologic mechanisms and treatment strategies is ongoing.
Short description of findings	Accidental exposure to small volumes of Roundup herbicide does not produce serious toxic effects (< 5 ml). Minor, local irritative effects are self limited and respond to basic first aid care. Large amounts ingested with suicidal intention place the patient at risk for toxic effects including gastrointestinal irritation, shock, pulmonary oedema, renal shutdown, and possibly death. Treatment is supportive.
Relevance of this literature article to the submission	This article describes the clinical findings and management of patients who present with a formulated glyphosate ingestion (Roundup) and may relate to the toxicity of surfactants used in specific formulations rather than the active ingredient.
RMS comments:	None

B.6.9.8.28. Literature study 28

Literature study 28 Wang et al. 2011

Data point	KCA 5.9.7
Report author	Gang Wang, Xiao-Ning Fan, Yu-Yan Tan, Qi Cheng, Sheng-Di Chen
Report year	2011
Report title	Letter to the Editor Parkinsonism after chronic occupational exposure to glyphosate
Report No	-
Document No	Parkinsonism and Related Disorders 17 (2011) 486–487
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	A patient with parkinsonism following chronic occupational exposure to glyphosate was reported. A previously healthy 44-year-old woman presented with rigidity, slowness and resting tremor in all four limbs with no impairment of short-term memory, after sustaining long term chemical exposure to glyphosate for 3 years as a worker in a chemical factory. The chemical plant produced a range of herbicides including: glyphosate, gibberellins, and dimethyl hydrogen phosphite; however, the patient worked exclusively in the glyphosate production division. She only wore basic protection such as gloves or a face mask for 50 h each week in the plant where glyphosate vapor was generated. She frequently felt weak. Two months before she came to the clinic, she had experienced severe dizziness and blurred vision. After being diagnosed by the local doctor with cervical spondylosis, the patient received treatment with DAN-SHEN (salvia) injections for one week without any improvement. There was no known family history of neurological or other relevant disorders. The patient had consumed no other medications or herbal preparations before the onset of symptoms.
Short description of findings	Not applicable, letter to editor.

Relevance of this literature article to the submission	This is a case report describing a patient who developed Parkinson's Disease and claiming that occupational exposure to glyphosate over 3 years was likely why the patient developed PD. Multiple GLP studies demonstrate that glyphosate is not neurotoxic. Additionally, Glyphosate cannot cross the blood brain barrier as it is hydrophilic and negatively charged. The article then goes on to state that glyphosate inhibits cholinesterase and other enzymes which demonstrates the fact that the authors are under the impression that glyphosate is an organophosphate, which is not the case. This patient also has atypical features on her MRI which suggest an entirely different PD aetiology.
Comments RMS	Agreed that glyphosate is not neurotoxic based on the available guideline studies. However, these studies do not investigate Parkinson's disease which is considered a complex and multifactorial disease. However, as this is only a single case in which the actual occupational exposure is not quantified, no conclusion can be drawn based on this case report. The study was also assessment by the former RMS. The evaluation is presented in the table below.

Reliability of study:	Not assignable
Comment:	Medical case report, single incident
Relevance of study:	Relevant with restrictions
Klimisch code:	4

B.6.9.8.29. Literature study 29

Literature study 29 Zheng et al. 2018

Data point	KCA 5.9.7
Report author	Qian Zheng, Jianhong Yin, Lina Zhu, Ling Jiao, Zhu Xu
Report year	2018
Report title	Correspondence Reversible Parkinsonism induced by acute exposure glyphosate
Report No	-
Document No	Parkinsonism and Related Disorders 50 (2018) 121
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	
GLP/Officially recognised testing facilities	-
Short description of literature article	On October 16, 2017, a previously healthy 58-year-old woman with a one day generalized headache without visual change came to be evaluated and was found to have a normal cranial MRI and lab studies. Tramadol hydrochloride did not help her headache. She developed progressive slowness and tremor of 4 limbs and difficulty in walking over the next several days. Fifteen days later she presented with a mask face, bradykinesia, cogwheel rigidity, markedly stooped posture with reduced arm swing, and a rest tremor in all limbs. MRI scan of the brain was again normal. There was no known family history of neurological disorders. She denied medications or herbal preparations before these symptoms. However she had been spraying glyphosate for over 3 hours per day for the week before the symptoms without using protective equipment. Glyphosate-induced Parkinsonian syndrome was suspected. She then underwent treatment with ATP, pralidoxime iodide and scopolamine hydrobromide and the headache and parkinsonism completely disappeared.
Short description of findings	Not applicable, letter to editor.
Relevance of this literature article to the submission	This is a case report that describes a patient who developed a reversible Parkinson Syndrome after glyphosate exposure. The symptoms resolved after the patient was treated with anticholinergic agents and oximes. This is the treatment for organophosphate poisoning and not that of glyphosate. Multiple GLP studies have demonstrated that glyphosate is not neurotoxic.
RMS comments:	The RMS considers that there is no causality between the clinical signs reported and the glyphosate-exposure as symptoms disappeared upon treatment with anticholinergic agents and oximes, which is a treatment for organophosphate poisoning.

B.6.9.8.30. Overview on case reports.

A list of case reports was submitted in the literature search report by the applicant (CA 9-01). The tables below provide the abstracts of the articles and an evaluation by the applicant.
The RMS is in agreement with the evaluation by the applicant, unless indicated in bold text in the table below.

Table B.6.9.8.30-1: Overview on case reports. No review information or epidemiologic information is listed in this table. The complete references on any kind of medical data are available in the literature search report, CA 9-01.

Reference	Evaluation by applicant	Abstract
Beswick, E. et al. (2011) Journal of the Intensive Care Society, (January 2011) Vol. 12, No. 1, pp. 37-39	This is a case report of a fatality after a formulated glyphosate suicidal overdose. Multiorgan failure is not uncommon in these overdoses. As this is a case report of a suicidal ingestion it should not impact re-registration decisions	A 29-year-old man was admitted following deliberate ingestion of approximately 300 mL of 'Roundup Ace,' a herbicide containing glyphosate. On presentation, he was agitated and required intubation and admission to ICU. He developed severe and persistent lactic acidosis, hyperkalaemia, hypotension, torrential watery diarrhoea and abdominal distension in the first 24 hours. The patient was supported with a continuous noradrenaline infusion and continuous veno-venous haemodiafiltration. The clinical course was further complicated by cardiac arrhythmias and an episode of cardiac arrest. By day three, he had bone-marrow, liver and worsening respiratory failure. There was no hope of recovery; therefore, noradrenaline was discontinued, and the patient died 15 minutes later. Glyphosate-surfactant herbicide (GlySH) is a general-purpose herbicide with no anticholinesterase effect and no organophosphate-like CNS effects. GlySH intoxication has a case fatality rate of between 3.2% and 29.3%. There is no antidote and the mainstay of treatment for systemic toxicity is decontamination and aggressive supportive therapy. Early renal replacement therapy may improve prognosis but there is no evidence to support this. There is one published case of a patient who survived severe GlySH poisoning following administration of intravenous fat emulsion. Glyphosate-surfactant herbicide, commercially known as 'Roundup' is a widely available herbicide commonly used in both professional and domestic settings. This is a case of a young man who deliberately ingested GlySH at home and rapidly developed multi-organ failure, culminating in his death.

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Bosak A. B. et al., 2014 Journal of Medical Toxicology (2014), Vol. 10, No. 1, pp. 72	This is a report about multi-organ failure after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Background: Glyphosate is a commonly used herbicide associated with toxicity and death following large ingestions. Surfactants are implicated as the primary contributor. Hypothesis: Glyphosate herbicide preparations contain various surfactants and salts which may lead to diverse clinical toxicity. Methods: (Case 1) A 50-year-old man presented with N/V, abdominal pain, and somnolence 12 h after ingesting 6.5 oz of concentrated Roundup ® containing 50.2 % glyphosate as isopropylamine salt. Labs revealed metabolic acidosis (AG 40), lactate (9.2 mmol/L), creatinine (2.8 mg/dL), potassium (4.5 mmol/L), WBC (41.5 K/mm3), and lipase (2,528 IU/L). Electrocardiogram QRS was 142 ms and responsive to bicarbonate. Patient became hypoxic and hypotensive requiring intubation, vasopres-sors, and continuous veno-venous hemodialysis (CVVHD) for anuric renal failure. A CT scan showed multiple loops of dilated small bowel and possible pneumatosis with negative exploratory surgery on day 1. On day 5, he had peritonitis prompting the resection of the ischemic terminal ileum and cecum. Over the next 3 weeks, he developed recurrent GI bleeds unrelated to surgery, remained anuric on CVVHD, and ventilator dependent. (Case 2) A 48-year-old landscaper ingested 250- 350 mL of concentrated glyphosate herbicide and presented with AMS and N/V. Labs showed WBC (21.2 K/mm3), potassium (7.1 mmol/L), metabolic acidosis (AG 10), and creatinine of 1.2 mg/dL (0.60 baseline). Electrocardiogram QRS was 192 ms with wide complex rhythm, bradycardia, and responsive to bicarbonate. Persistent hyperkalemia continued despite medical treatment requiring high flux hemodialysis. He required ventilator support for hypoxia and had a negative EGD. Results: Case 1 developed anuric renal failure, QRS prolongation, ischemic bowel, and recurrent GI bleeds. Care was withdrawn on day 25. Case 2 had persistent hyperkalemia, non-anuric renal failure, QRS prolongation, and bradycardia. Discussion: We hypothesize that case 2 ingested a potassium salt preparation due to the persistent hyperkalemia that only resolved with hemodialysis. QRS prolongation was present in both cases responsive to bicarbonate. Conclusion: Glyphosate containing herbicides may have diverse clinical toxicity depending on surfactant and salt preparation.

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Chan C-W. et al., 2016 Critical care medicine (2016), Vol. 44, No. 1, pp. E45	This paper looked at the use of ECMO in a critically ill patient after formulated glyphosate product overdose. ECMO is sometime of utility in treating overdose patients. This paper should not impact re-registration.	OBJECTIVE: To describe the experience of emergency extracorporeal membrane oxygenation in treating life-threatening glyphosate-surfactant intoxication. DESIGN: Case report. SETTING: Emergency department and ICU. PATIENT: A patient with cardiopulmonary failure after glyphosate-surfactant intoxication. INTERVENTION: Extracorporeal membrane oxygenation. CASE REPORT: A 47-year-old man presented with mildly decreased consciousness in our emergency department after ingesting approximately 100 mL of glyphosate-surfactant 1.5 hours previously. Respiratory failure, persistent ventricular tachycardia, profound shock refractory to inotropic agents, and metabolic acidosis developed in the patient within 2 hours. Extracorporeal membrane oxygenation was applied within 4 hours of cardiopulmonary failure. The patient's condition improved considerably. He was transferred to the general ward on the eighth day with stable hemodynamic status and complete neurological recovery. CONCLUSIONS: On the basis of our research, this was the first case in which extracorporeal membrane oxygenation was used to treat severe glyphosate-surfactant intoxication. We recommend early initiation of extracorporeal membrane oxygenation therapy to mitigate cardiopulmonary compromise in patients with glyphosate-surfactant intoxication.
Cho, Y. et al. (2019) AMERICAN JOURNAL OF EMERGENCY MEDICINE, (AUG 2019) Vol. 37, No. 8.	This case report describes the successful treatment of hyperkalemia and monitoring of blood glyphosate concentrations after a formulated glyphosate overdose. It is expected that the glyphosate level would be high on admission and then decrease rapidly. This is a case report of a suicidal ingestion and should not impact re-registration.	Introduction: This report describes changes in blood and urine concentrations of glyphosate potassium over Lime and their correlations with clinical symptoms in a patient with acute glyphosate potassium poisoning. Case report: A 67-year-old man visited the emergency center after ingesting 250 mL of a glyphosate potassium based herbicide 5 h before. He was alert but presented with nausea, vomiting, and bradyarrhythmia with atrial fibrillation (tall T waves). Laboratory findings revealed a serum potassium level of 6.52 mEg/L. After treatment with an injection of calcium gluconate, insulin with glucose, bicarbonate, and an enema with polystyrene sulfonate, the patient's serum potassium level normalized and the bradyarrhythmia converted to a normal sinus rhythm. During admission, the blood and urine concentration of glyphosate and urine aminomethylphosphonic

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		acid (AMPA, a glyphosate metabolite) was measured at regular time intervals. The patient's glyphosate blood concentration on admission was 11.43 mg/L, and it had decreased rapidly by 16 h and maintained about 1 mgl/L by 70 h after admission. Urine glyphosate and AMPA levels had also decreased rapidly by 6 h after admission. Discussion: Glyphosate potassium poisoning causes hyperkalemia. Blood concentrations of glyphosate were decreased rapidly by 16 h after admission, and urine concentrations were also decreased by 6 h after admission. (C) 2019 Elsevier Inc. All rights reserved.
Choi B. et al., 2013 Toxicology Letters (2013), Vol. 221, Supp. 1, pp. S66	This is a report about measuring IMA rather than lactate as a marker of shock after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Introduction: To date, plasma lactate level has been thought that the most important monitoring tool to measure systemic tissue hypoxia. However, the time lag and relatively low value in some cases are frequently encountered. Case: Sixty-seven year old female was admitted emergency room after 80 min from acute pesticide poisoning. She drank 500mL of glyphosate with intension of suicide. She was alert but her vital signs were unstable at admission; BP 84/37mm Hg, PR 97/min, SpO2 98%. After the initial resuscitation management including general decontamination treatment, she moved to the Acute Care Unit and continuous resuscitation due to her unstable hemodynamic status. During the hospital course, we daily measured the ischemic modified albumin (IMA) level using albumin-cobalt binding assay that its value could tell us the tissue hypoxia. Among the monitoring values, the trend of IMA and base deficit would be correlated with the clinical progress (Fig. 1). Conclusion: We experienced that IMA has a more sensitive monitoring value than lactate in critically ill patient in our acute pesticide poisoning patient. IMA could be measured in venous blood and may be an alternative monitoring laboratory value as base deficit. Also, further study is warranted.
De Raadt W. M. et al., 2015 Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG (2015), Vol. 32, No. 2, pp. 172	This article is a case report of a smoker who developed eosinophilic pneumonia after glyphosate exposure. Glyphosate is not a sensitizer as established by multiple GLP regulatory studies. Nozzle application of formulated glyphosate produces aerosols of between 200-350 microns. In humans, it takes droplets of <100 microns to cause inhalational injury. The claim that formulated glyphosate can cause inhalational injury in a setting where	We report a case of a female patient who developed acute eosinophilic pneumonia (AEP) after recent onset of smoking and exposure to glyphosate-surfactant. The additional exposure associated with the recent start of smoking may have contributed to the development and/or severity of AEP. A clinical relapse after re-challenge four years later both with smoking and glyphosate-surfactant made the association highly likely. Respiratory distress is a factor of poor outcome and mortality after ingestion of glyphosate-surfactant. This case highlights the importance of a thorough exposure history e.g., possible

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	it isn't aspirated is not biologically plausible. **Remark RMS: agree that glyphosate is not a sensitizer. However, we disagree that inhalation is not possible as no information is available on how the product was applied and whether or not small droplets can be excluded. On the other hand, the case is related to a glyphosate-based product and not on glyphosate alone, therefore it is not possible to to distinguish if they were due to the active substance or rather to co-formulants.**	occupational and environmental exposures together with drug-intake. Genotyping should be considered in cases of severe unexplained pulmonary damage.
Deo S. P. et al., 2012 Journal of the Nepal Medical Association (2012), Vol. 52, No. 185, pp. 40	Large ingestions of formulated glyphosate can often result in caustic injury secondary to the surfactant's detergent actions on the mucous membranes of in people who ingest them. That said, they shouldn't cause microstomia, which tends to result from much more corrosive and scarring chemicals. This should not impact re-registration.	Glyphosate (GlySH) is a broad spectrum, nonselective herbicide, widely used in agriculture. This case report describes a 25-year-old man presenting with extensive chemical burns and ulceration of the oral cavity as a result of accidental exposure to GlySH. This paper aims to illustrate the typical appearance of GlySH related chemical mucosal burn and to demonstrate the severity of the corrosive effect of GlySH which need team approach to prevent unfavourable sequelae such as microstomia.
Elsner, P. (2018) Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG, (2018 Jan) Vol. 16, No. 1, pp. 70-71	This paper describes a patient who spilled formulated glyphosate on her arms then washed it off. A week later she developed psoriasis which spread from her arms to her body. She filed a medicolegal claim which was then reported to this journal as a correspondence. The MD wrote that he reviewed the 1986 Maibach paper which evaluated >300 volunteers and showed no evidence of irritation or sensitization and countered this study with 2 case reports alleging sensitizatioin, that the psoriasis on the patient's hands and arms was related to glyphosate exposure, whereas the rest of the psoriasis on her body was not related to the exposure. There is not a mechanism for glyphosate to cause psoriasis, especially 1 week post exposure.	Not available

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Reference	Evaluation by applicant	Abstract
Frappart, M. (2011) Vol. 30, No. 11, pp. 852-4.	This case report describes caustic injury to the GI tract and multiorgan failure after formulated glyphosate overdose. The clinical course is consistent with previous reports of overdose and should not impact re-registration.	Not available
Garlich F. M. et al., 2014 Clinical toxicology (2014), Vol. 52, No. 1, pp. 66	This article discusses the successful use of haemodialysis in a patient who was critically ill after a formulated glyphosate overdose.	CONTEXT: Ingestion of glyphosate-surfactant herbicides (GlySH) can result in acute kidney injury, electrolyte abnormalities, acidosis, cardiovascular collapse, and death. In severe toxicity, the use of hemodialysis is reported, but largely unsupported by kinetic analysis. We report the dialysis clearance of glyphosate following a suicidal ingestion of a glyphosate-containing herbicide. CASE DETAILS: A 62-year-old man was brought to the emergency department (ED) 8.5 h after drinking a bottle of commercial herbicide containing a 41% solution of glyphosate isopropylamine, in polyoxyethyleneamine (POEA) surfactant and water. He was bradycardic and obtunded with respiratory depression necessitating intubation and mechanical ventilation. Initial laboratory results were significant for the following: pH, 7.11; PCO2, 64 mmHg; PO2, 48 mmHg; potassium, 7.8 mEq/L; Cr 3.3, mg/dL; bicarbonate, 22 mEq/L; anion gap, 18 mEq/L; and lactate, 7.5 mmol/L. Acidosis and hyperkalemia persisted despite ventilation and fluid resuscitation. The patient underwent hemodialysis 16 h post ingestion, after which he demonstrated resolution of acidosis and hyperkalemia, and improvement in clinical status. Serum glyphosate concentrations were drawn prior to, during, and after hemodialysis. The extraction ratio and hemodialysis clearance were calculated to be 91.8% and 97.5 mL/min, respectively. DISCUSSION: We demonstrate the successful clearance of glyphosate using hemodialysis, with corresponding clinical improvement in a patient with several poor prognostic factors (advanced age, large volume ingested, and impaired consciousness). The effects of hemodialysis on the surfactant compound are unknown. Hemodialysis can be considered when severe acidosis and acute kidney injury complicate ingestion of glyphosate-containing products.
Han Sang Kyoon et al. (2010) Vol. 48, No. 6, pp. 566-8.	This is a case report of a suicidal ingestion of formulated glyphosate that was treated with lipid emulsion and improved. Since this is a description of medical management of a suicidal overdose, this should no impact re-registration	CONTEXT: Circulatory shock is a major cause of mortality in glyphosate-surfactant herbicide (GlySH) poisoning, and this condition responds poorly to conventional therapies. We report a case of GlySH poisoning with shock that was refractory to vasopressors but responsive to intravenous fat emulsion (IFE). CASE DETAILS: A 52-year-old man was brought to the emergency department

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		by ambulance. He was found unconscious in his living room along with an empty bottle of GlySH herbicide, which contained glyphosate, polyoxyethyleneamine (POEA) surfactant, and water. He was drowsy at presentation. His heart rate was 44 beats/min, his blood pressure could not be measured with an arm cuff, but he had a palpable femoral pulse. After about 2.5 h of supportive care after admission, he remained hypotensive, and his systolic blood pressure was 80 mmHg. A 500 mL bottle of 20% IFE product was prepared. As a bolus, 100 mL of IFE was injected, and the remaining 400 mL was then infused. His blood pressure was 100/60 mmHg 1 h after the bolus injection. At 5 h after IFE injection, his blood pressure reached 160/100 mmHg and vasopressors were tapered. CONCLUSION: IFE should be considered in cases of refractory hemodynamic instability caused by GlySH after aggressive fluid and vasopressor support.
Hansen N. B. et al., 2013 Clinical Toxicology (2013), Vol. 51, No. 4, pp. 354	This is a case report of an accidental ingestion of formulated glyphosate resulting in mild corrosive injury to the GI tract in a small child and should not impact re-registration.	Objective: Glyphosate is a broad-spectrum systemic herbicide. Severe toxicity is rare. Case report: A 2.5 year-old boy ingested unknown amounts of concentrated glyphosate (Round-Up), decanted into a soda bottle. Immediately after ingestion the boy was crying, experienced excessive salivation, vomited several times, spontaneous and provoked. Fifteen minutes after ingestion the National Poison Center was contacted, and advised immediate emergency ward contact. The Poison Center alerted the hospital staff about the patient and treatment plan including activated charcoal and examination of oral and gastrointestinal mucosa. On emergency ward arrival the child was awake, crying, reacted adequately, was warm and dry, respiratory frequency (RF) 19/min, oxygen saturation 100%, heart rate 164 beats/min and blood pressure 104/71 mmHg. Activated charcoal 1 g/kg (body weight 14.5 kg) was dosed 1 hour after ingestion. Corrosive injury was suspected because of hypersalivation and red marks in the mouth and throat. Gastroscopy 3 hours after ingestion showed Grade 1b ulceration in the esophagus, pylorus and gastric mucosa. Antibiotic treatment (cefuroxime and metronidazole), with antiemetic (dexamethasone), and analgesics (diclofenac and paracetamol), was initiated. A gastric tube was placed for observation. Hemorrhage was not observed. Coagulation parameter deterioration with elevated international normalized ratio (INR 1.4) and decreased activated partial thromboplastin time (APTT 25 sec), was observed from 3 hours after ingestion

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		and persisted to day 4. All other biochemical parameters were within normal range, and there was no sign of acute kidney injury. Three days after ingestion soft diet was initiated and the following day the patient was well and discharged. Conclusion: Typical symptoms from glyphosate ingestion include nausea, vomiting, abdominal pain, mouth and throat pain. In severe cases gastrointestinal ulceration and bleeding, renal and hepatic deterioration, and circulatory effects might be observed. There have been deaths after rapid onset of respiratory and circulatory collapse. In this case the ingested amount is unknown but is supposed to be a limited minor amount of a highly concentrated product. Immediate development of symptoms and gastric mucosa ulceration confirmed by gastroscopy support the suspicion of concentrated glyphosate ingestion. Unfortunately it was not possible to get a product sample for chemical analysis. The effect on coagulation parameters was unexpected and calls for increased attention in future glyphosate poisonings.
Iwai K. et al., 2014 Journal of Clinical Toxicology (2014), Vol. 4, No. 6, pp. 1	This article discusses the use of endoscopy to treat formulated glyphosate overdose and medical management of suicidal ingestions and therefore should not impact registration decisions.	Introduction: Roundup® is a herbicide widely used in Japan in gardening and agriculture. When ingested, Roundup is highly toxic, but gastrointestinal decontamination, including gastric lavage, is not routinely performed after ingestion. Endoscopy may be useful in managing individuals with liquid herbicide poisoning, by identifying gastric residual contents, assessing mucosal damage and retrieving herbicide directly by aspiration. Case report: A 73 year old, 40 kg female with a history of depression was transported to our emergency room by ambulance 1 h after attempting suicide by ingesting 100 ml Roundup, which contains 48% glyphosate-potassium, and 52% surfactant and water. This volume was below a fatal dose (<5000 mg/kg), but may have caused organ dysfunction and mucosal damage. After confirming respiratory and circulatory stability and after obtaining informed consent from the patient, endoscopy (XQ 260; Olympus, Tokyo, Japan) was performed in the emergency room to retrieve residual herbicide. About 80 ml of herbicide in the stomach were aspirated endoscopically with only mild erosion observed in the mucosa of the stomach. The patient was able to resume oral intake 2 days after endoscopy and was discharged without any complications on day 5. Conclusion: Endoscopy may be useful in cases of liquid poisoning including, Roundup, both to determine the amount of residual toxin and to remove it from the stomach.

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Jovic-Stosic J. et al., 2013 Clinical Toxicology (2013), Vol. 51, No. 4, pp. 288.	This is a case series that included one patient with a formulated glyphosate overdose and treatment with ILE. This describes medical management of overdoses and should not impact re-registration.	Objective: To assess the efficacy and complications of intravenous lipid emulsion (ILE) antidotal use in acute human poisoning. Methods: Prospective clinical study on ILE (Intralipid 20%) effects given as fast intravenous infusion in total dose of 500- 1000 mL. The main criteria for administration were cardiocirculatory failure caused by liposoluble agents and poor response to vasopressors. Effects on blood pressure (BP), electrocardiogram (ECG), and central nervous system (CNS) depression were assessed. Pre- and post-lipid administration concentrations of drugs were obtained. Results: A total of nine patients were treated with ILE. Poisonings were caused by glyphosate/polyethyloxylated tallowamine (POEA) herbicide (1 patient), verapamil and benzodiazepines (3 patients), propranolol combined with alcohol or psychoactive drugs (2 patients) and mixed ingestion of various drugs including carbamazepine, lamotrigine, sertraline, risperidone, amitriptyline, clozapine, haloperidol, valproic acid/valproate and chlorpromazine (3 patients). Significant increase of BP leading to vasopressor therapy reduction was noted in all patients after the initial dose of 500 mL, but in some cases this effect was transient and an additional dose of Intralipid was necessary. The most prominent effect was on wide complex tachycardia which developed in two patients (ingested glyphosate/POEA or propranol/alcohol) as sinus rhythm was regained before the end of Intralipid infusion. ECG changes in others included slight widening of QRS or QT prolongation. There was no rapid normalisation that could be attributed to ILE. All patients were comatose (Glasgow Coma Scale (GCS) 3-5). Improvement in GCS was noted in all except the cases with predominant carbamazepine and valproate. There were no significant changes in drug concentrations in blood after lipid administration. In cases of verapamil toxicity, analysis after lipid removal by ultracentrifugation revealed a decrease in concentration. The only complication which may be connected with ILE treatment was acute respiratory distress syndrome (ARDS) in a case of severe verapamil intoxication. Conclusion: Our results, though limited by the small number of patients, revealed that the most invariable effect of ILE was the increase of BP, and the most impressive was the fast reversal of wide complex tachycardia. Efficacy of ILE in case of poor response to other therapies indicated that the fear of adverse interactions with other conventional drugs for the

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		treatment of cardiotoxicity may not be rational.
Jovic-Stosic J. et al., 2016 Vojnosanitetski pregled (2016), Vol. 73, No. 4, pp. 390	Medical case of intentional ingestion. ILE has been proposed as a possible therapy for formulated glyphosate overdoses. As this was a suicide attempt, this should not impact re-registration.	Introduction: Glyphosate is the first widely used herbicide against weed in genetically modified crops. Though glyphosate itself has a low toxicity, commercial products are more dangerous because of increased toxicity due to surfactants addition. There is no specific antidote for the poisoning with glyphosate-surfactant (Gly-SH). In recent times, the efficacy of intravenous lipid emulsion (ILE) administration for the treatment of acute poisoning caused by Gly- SH has been investigated. Case Report: A 50-year-old man was admitted 3 hours after self-poisoning with herbicide containing glyphosate and polyoxyethyleneamine, as a surfactant. On admission, the patient was in a coma, hypotensive (80/50 mmHg) and without spontaneous breathing. Electrocardiogram showed widecomplex tachycardia, and arterial blood gas (ABG) revealed acidosis (pH 7.07). Conventional treatment included mechanical ventilation, intravenous fluids, bicarbonate and dopamine. As there was no improvement, ILE was started. The patient received 100 mL of 20% Intralipid® bolus followed by infusion of 400 mL over 20 minutes. Prior to expiration of infusion, a gradual rise in blood pressure was noted, and within 2 hours sinus rhythm was restored. Conclusion: This case report suggests that the use of ILE may be an additional option for the treatment of cardiocirculatory disturbances caused by commercial products of glyphosate herbicide.
Jovic-Stosic J. et al., 2016 Clinical Toxicology (2016), Vol. 54, No. 4, pp. 476.	This is a report about using ILE to treat overdoses with 1 patient who ingested formulated glyphosate. This paper should not impact re-registration.	Objective: Intravenous lipid emulsion (ILE) may successfully resuscitate patients with cardiotoxicity. However, the indications for its use, as well as its efficacy, are not sufficiently defined. Methods: An observational clinical study on the effects of lipid (Intralipid 20%) given as an intravenous infusion to a total dose of 500-1000 mL. The main criteria for administration of ILE were cardiocirculatory failure caused by liposoluble chemicals (drugs or pesticides) and poor response to conventional treatment which included dopamine, glucagon, bicarbonate and calcium chloride. Effects on blood pressure (BP), electrocardiogram (ECG) and survival of patients were assessed. Results: There were 31 patients (aged 28-83 years) treated with ILE, which comprised approximately 1% of the total number

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		of patients hospitalized in the intensive care unit due to poisoning during 5-year follow up. ILE was most frequently used in calcium channel blockers poisoning (15 patients). In 9 patients who had ingested verapamil, or a combination of verapamil and benzodiazepines or angiotensin converting enzyme (ACE) inhibitors (enalapril, cilazapril), ILE was effective in reversing hypotension and dysrhythmias. There were 5 cases of multi-drug poisoning including amlodipine. Lethal outcome occurred in a patient poisoned by a combination of amlodipine with cilazapril, metformin and gliclazide. Combination of nifedipine and metoprolol was fatal despite treatment including ILE and pace-maker administration. Beta-blockers, including propranolol, metoprolol and bisoprolol were among the ingested drugs in 7 cases. Metoprolol was also lethal in combination with gliclazide, mianserin and benzodiazepines. Administration of ILE rapidly improved conduction delay and increased BP in cases of propafenone (1 patient) and glyphosate herbicide (1 patient) toxicity. In two cases of organophosphate insecticide poisoning with cardiovascular collapse, only transient increase of BP was noted. The remaining cases involved psychoactive drugs. ILE was successful in the treatment of clomipramine, maprotiline, sertraline and risperidone overdoses, but failed to reverse cardiotoxicity in patients who ingested carbamazepine, lamotrigine or valproate. Conclusion: Although all our patients received multiple therapies, the improvement observed in most of them soon after administration ILE can be attributed to its beneficial effects. Our experience revealed that the most invariable result of ILE administration was an increase in BP. The most impressive effect was the fast reversal of wide complex tachycardia in 3 different cases (ingestion of propranolol, glyphosate and propafenone) which may suggest effectiveness of ILE in the treatment of sodium channel blockade. However, ILE was not effective in all cases in which it could be expected on the basis of the toxic agent's liposolubility.
Jyoti W. et al., 2014 Journal of postgraduate medicine (2014), Vol. 60, No. 3, pp. 346	Formulated glyphosate can cause caustic injury to the mucosa membrane after ingestion. The esophagus is especially prone to perforation. Due to the absence of a serosa, the esophagus is notoriously difficult to repair & heal. This is not an unusual feature of caustic injury. As	Not available

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	this was a suicide attempt, this should not impact re-registration.	
Kato Y., 2015 The Japanese journal of toxicology (2015), Vol. 28, No. 4, pp. 368	This article describes a case series of three patients who presented with extreme hyperkalemia after suicidal ingestion of formulated glyphosate. This is not unexpected in an ingestion involving glyphosate formulated product with potassium salts and should not affect re-registration.	Not available
Knezevic V. et al., 2012 Srpski arhiv za celokupno lekarstvo (2012), Vol. 140, No. 9-10, pp. 648	Glyphosate based formulations can cause renal injury in overdose, and the K+ formulations may result in hyperkalemia. It is therefore reasonable to start hemodialysis or hemofiltration in critically ill patients with kidney failure or hyperkalemia. As this was a suicide attempt, this should not impact re-registration.	INTRODUCTION: Treating severe acute glyphosate-surfactant poisoning requires intensive therapy including dialysis. Cases of hemoperfusion and hemodialysis use in renal failure induced by herbicide ingestion have been reported in the current medical literature. We present a case report of successful patient treatment with continuous veno-venous hemodiafiltration in acute glyphosate-surfactant poisoning. CASE OUTLINE: A 36-year-old male patient attempted suicide by drinking approximately 300 ml of glyphosate-surfactant about an hour before coming to our Clinic. On admittance the patient was somnolent, normotensive, acidotic and hyperkalemic. Six hours after poison ingestion there was no positive response to symptomatic and supportive therapy measures. The patient became hypotensive, hypoxic with oliguric acute renal failure, so that post-dilution continuous veno-venous hemodiafiltration was started. During the treatment the patient became hemodinamically stabile, diuresis was established along with electrolyte and acid-base status correction and a gradual decrease of blood urea nitrogen and creatinine levels. After a single 27.5-hour treatment, clinical condition and renal function parameters did not require further dialysis. Complete recovery of renal function was achieved on the fifth day. CONCLUSION: Early introduction of continuous veno-venous hemodiafiltration with other intensive therapy measures led to complete recovery in a hemodinamically instable patient.

Table B.6.9.8.30-1: Overview on case reports. No review information or epidemiologic information is listed in this table. The complete references on any kind of medical data are available in the literature search report, CA 9-01.

Reference	Evaluation by applicant	Abstract
Lee, G. (2018) Journal of Veterinary Clinics (2018), Volume 35, Number 4, pp. 144-145, 10	Multiple GLP studies have been conducted to evaluate whether glyphosate is neurotoxic. There is no mechanism by which glyphosate can cross the Blood Brain Barrier or enter the bundles of neurons in the cauda equina to cause a lower motor neuron lesion. Given the clinical description, this sounds more like a Guillan-Barre syndrome which has been reported in dogs.	A 4-year-old Yorkshire terrier was presented with hindlimb paresis and urinary incontinence after accidental ingestion of an herbicide. Based on neurologic examinations, decreased hindlimb proprioception with flaccid paresis were revealed. Other possible causes of the clinical signs were excluded. The clinical signs gradually improved after administration of anti-inflammatory and antioxidant therapy. This case report is the first to describe the long-term outcome of hindlimb paresis and urinary incontinence induced by glyphosate surfactant herbicide (GPSH) poisoning in a dog.
Lee B. K. et al., 2012 Hong Kong Journal of Emergency Medicine (2012), Vol. 19, No. 3, pp. 214	This is a report about multi-organ failure and the use of CVVHD after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Intractable hypotension is a major cause of death after glyphosate-surfactant herbicide poisoning. However, there is no specific treatment besides conservative care. Herein, we report a patient poisoned by glyphosatesurfactant herbicide experiencing cardiac arrest but was successfully resuscitated and treated with continuous venovenous haemodiafiltration (CVVHDF). The 60-year-old patient was brought to our emergency department after ingesting glyphosate-surfactant herbicide. He developed pulmonary oedema, severe metabolic acidosis (pH 6.960), and hyperkalaemia (serum potassium 8.8 mmol/L). Although he experienced cardiac arrest for about 12 minutes, the use of CVVHDF improved the metabolic acidosis and hyperkalaemia, and finally stabilised his vital signs. He regained an alert mental state after therapeutic hypothermia. CVVHDF, which is a better tolerated renal replacement therapy than haemodialysis in haemodynamically unstable patients, should be considered in glyphosate-surfactant poisoned patients of intractable hypotension with severe metabolic acidosis or hyperkalaemia..
Lee D. H. et al., 2017 Hong Kong Journal of Emergency Medicine (2017), Vol. 24, No. 1, pp. 40	This is a report about multi-organ failure and the use of dialysis after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Glyphosate-surfactant is one of the most commonly used herbicides in the world. Its key component, glyphosate, is a competitive inhibitor of the shikimate pathway, a metabolic pathway found only in plants. However, severe intoxication, including lethal cases by ingestion of, glyphosate-surfactant has been reported. We describe the full recovery of two patients from glyphosate-polyoxyethyleneamine surfactant intoxication and multi-organ system failure following continuous renal replacement therapy. Both patients developed persistent shock, acute kidney injury, lactic acidosis, hyperkalaemia and multi-organ failure despite of resuscitation. We believe that continuous renal replacement therapy should be initiated immediately for removal of glyphosate-

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Reference	Evaluation by applicant	Abstract
		polyoxyethyleneamine surfactant in patients with signs of cardiopulmonary compromise, lactic acidosis, and renal failure. We propose the addition of glyphosate-polyoxyethyleneamine surfactant to the list of toxins for which early haemodialysis should be indicated.
Luo W. et al., 2019 Medicine (2019), Vol. 98, No. 30, pp. e16590	This article describes a case report of gastric ulceration and swelling causing pyloric obstruction in a patient who ingested formulated glyphosate. This is not unexpected as formulations contain surfactants which can cause caustic injury to the GI tract with suicidal ingestions. This should not impact re-registration.	RATIONALE: Oral ingestion of glyphosate can induce gastrointestinal symptoms such as vomiting, abdominal pain, and hematochezia. Timely and effective treatment of pyloric stenosis caused by glyphosate poisoning is important. PATIENT CONCERNS: The patient had a poor appetite, accompanied by nausea and vomiting of a small amount of dark brown material that resembled blood clots several times a day. Gastroscopy revealed gastric ulcer, a large pyloric antrum ulcer, and a deformed stomach cavity. DIAGNOSIS: Pyloric stenosis due to glyphosate poisoning in a 36-year-old man. INTERVENTIONS: The patients received distal gastrectomy and subsequently transferred to the ICU for further treatment. A mechanical ventilator was used to assist breathing. OUTCOMES: Follow-up was conducted 3 years after surgery. The patient had no problem with food ingestion and experienced no discomfort, such as vomiting, nausea, coughing, or expectoration. LESSONS: Gastrectomy is necessary to treat pyloric stenosis caused by glyphosate poisoning.
Mahendrakar K. et al., 2014 Indian journal of critical care medicine (2014), Vol. 18, No. 5, pp. 328	ILE has been proposed as a possible therapy for formulated glyphosate overdoses.	Glyphosate is a widely used herbicide in agriculture, forestry, industrial weed control and aquatic environments. Glyphosate potential as herbicide was first reported in 1971. It is a non-selective herbicide. It can cause a wide range of clinical manifestations in human beings like skin and throat irritation to hypotension, oliguria and death. We are reporting a case of a 35-year-old male patient who was admitted to our tertiary care hospital following intentional ingestion of around 200 ml of herbicide containing glyphosate. Initially, gastric lavage done and the patient was managed with intubation and mechanical ventilation, noradrenaline and vasopressin infusion, continuous veno-venous hemodiafiltration and intravenous (IV) lipid emulsion (20% intralipid 100 ml), patient was successfully treated and discharged home. This case report

Table B.6.9.8.30-1: Overview on case reports. No review information or epidemiologic information is listed in this table. The complete references on any kind of medical data are available in the literature search report, CA 9-01.

Reference	Evaluation by applicant	Abstract
		emphasizes on timely systemic supportive measure as a sole method of treatment since this poison has no known specific antidote and the use of IV lipid emulsion for a successful outcome.
Malhotra, R.C. (2010)	This paper describes prolonged encephalopathy in a suicidal glyphosate ingestion. There is no mention of the medication that was used for sedation while the patient was intubated in the ICU. Accumulations of lorazepam and other sedatives may result in prolonged coma. In formualted glyphosate overdose with multiorgan failure it is common to sedate patients until their hemodynamics improve. In the setting of suicidal overdose, this paper should not impact re-registration.	Glyphosate-surfactant (GlySH) is a commonly used herbicide that has been used in attempted suicide. Most reports of GlySH toxicity in patients have followed ingestion of the commercial product "Round-up" (Monsanto Ltd; Melbourne, Victoria, Australia), which consists of a mixture of glyphosate (as a isopropylanine salt) and a surfactant (polyoxyethyleneamine). Ingestion of Round-up is reported to cause significant toxicity including nausea, vomiting, oral and abdominal pain. Renal and hepatic impairment and pulmonary oedema may also occur. Impaired consciousness and encephalopathy have been reported as sequelae but there are limited data on the central nervous system (CNS) effects of Round-up toxicity. We report a 71-year-old male who attempted suicide with GlySH and developed a prolonged but reversible encephalopathy suggestive of acute CNS toxicity. Copyright © 2010 Elsevier Ltd. All rights reserved.
Nakae H. et al., 2015 Acute medicine & surgery (2015), Vol. 2, No. 3, pp. 214	This article describes alternative medicine therapies that were used to treat a Japanese woman with a paralytic ileus after glyphosate ingestion. It is not uncommon for patients in a critical care setting to develop an ileus. These tend to resolve on their own without intervention. I cannot be commented on whether this intervention increases GI motility.	Case: A 65-year-old woman ingested glyphosate-surfactant herbicide in an attempt to commit suicide. She experienced glyphosate intoxication associated with multiple organ failure and developed a paralytic ileus. Daijokito, a traditional Japanese Kampo medicine was given to the patient to improve constipation and psychological symptoms. Next, rikkunshito was given to increase her gastric motility. Finally, daikenchuto was given to improve overall digestive peristalsis. Outcome: All abdominal symptoms ultimately improved after treatment with daikenchuto. Conclusion: Kampo medicines may help improve abdominal symptoms associated with glyphosate intoxication in cases where modern medical treatment alone proves inadequate.
Nakayama T. et al., 2019 Clinical and experimental nephrology (2019), Vol. 23, No. 6, pp. 865	This was a suicidal ingestion of formulated glyphosate that resulted in poor renal perfusion & multiorgan failure. Since this was a suicidal ingestion, the outcome is not unexpected and should not impact the re-egistration.	A 70-year-old woman presented with abdominal pain 2 h after ingesting 500 ml of glyphosate–surfactant herbicide (GPSH). As her abdominal pain worsened, contrast-enhanced computed tomography was performed 12 h after the admission, demonstrating renal blood flow shunting through the medulla which is called “reverse rim sign” (Fig. 1). She had been anuric since hospitalization with sufficient mean artery pressure and negative blood culture. Despite intensive

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Reference	Evaluation by applicant	Abstract
		care, she died of multiple organ failure on the 6th day of hospitalization. [No abstract was available; this is copied from article. MSB]
Ozaki T. et al., 2017 Therapeutic apheresis and dialysis (2017), Vol. 21, No. 3, pp. 296	This article discusses the use of haemodialysis and haemofiltration in formulated glyphosate overdoses. This article discusses medical management of suicidal ingestions and therefore should not impact registration decisions.	INTRODUCTION: In our case report we describe the case of a patient who experienced a stroke in her left hippocampus that was found following an attempted suicide via glyphosate overdose. To the best of our knowledge this is the first case report to describe a hippocampal infarction associated with a drug overdose. CASE PRESENTATION: A 64-year-old Japanese woman was brought to our emergency department after ingestion of an unknown dose of glyphosate surfactant herbicide in order to attempt suicide. On admission, she was assumed to be presenting with depression or psychiatric illness, however, sudden-onset memory deficit became apparent. The patient manifested delirium, confusion, and severe anxiety. In addition, short-term memory loss was prominent, with the patient forgetting her attempted suicide. Following an array of standard tests and a brain computed tomography scan (which only showed an old infraction), we performed a magnetic resonance imaging scan and neuropsychological evaluations. The brain magnetic resonance image revealed a small high-intensity lesion in the dorsal part of the left hippocampal body, and memory tests demonstrated severe short-term recall deficits. We diagnosed her with a left hippocampal infarction and administered a course of 75mg of clopidogrel. She gradually became less confused over the course of a week, and a follow-up memory test revealed partial improvement in some domains. No abnormalities were found on a follow-up brain scan. However, despite rehabilitation, memory impairments remain. CONCLUSIONS: It is important to note that had the symptom of short-term memory been absent or less severe, she might have been misdiagnosed with depression or another psychiatric illness. Although a computed tomography scan failed to detect hippocampal lesions, a diffusion-weighted magnetic resonance imaging scan clearly revealed a lesion within the left hippocampus. Therefore, in addition to assessments focusing on psychiatric illnesses that might be the root cause of an attempted suicide, organic factors should be considered along with radiological examination and precise memory assessments for diagnosing similar

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Reference	Evaluation by applicant	Abstract
		cases.
Picetti E. et al., 2017 Acta Biomedica (2017), Vol. 88, No. 4, pp. 533	This is a report about multi-organ failure after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Not available
Thakur D. S. et al., 2014 Toxicology international (2014), Vol. 21, No. 3, pp. 328	This is a case report of the clinical manifestations of glyphosate-based herbicide ingestions and discusses predictors of mortality in suicidal ingestions and therefore should not impact registration decisions.	Background: Water soluble and insoluble chemicals in the pesticide formulation may be eliminated more effectively in time if hemodialysis (HD) and hemoperfusion (HP) are performed concurrently. Aim: This study is aimed at evaluating the efficacy of concurrent HP and HD in patients with acute pesticide intoxication. Methods: Between January 2011 and December 2012, we used HP and HD consecutively (HP-HD group, 347 cases), and then during the next 2 years (January 2013 to December 2014), we used concurrent HP and HD (HPD group, 383 cases). We compared the clinical outcomes between the 2 groups. Results: The mortality was higher in the HP-HD group than in the HPD group: (48.1 vs. 20.9%) for the overall mortality and (81.8 vs. 57.9%) for the paraquat (bipyridylium) mortality ($p < 0.001$). In multiple logistic analyses, age ($p = 0.013$), ingested volume ($p < 0.001$), and HP-HD ($p = 0.014$) were significant risk factors for mortality in the paraquat ingested group. Conclusion: Concurrent HP and HD would be an effective and safe treatment for patients with acute pesticide intoxication, in particular, paraquat intoxication.
Veale D. J. H. et al., 2013 SAMJ (2013), Vol. 103, No. 5, pp. 293	This article summarises the chemicals used in South Africa for suicide. Glyphosate is only mentioned in a table in the article as being involved in 23 cases over a 1 year period accounting for 0.9% of the overall cases reported.	A 55 years old man self-presented to our Emergency Department (ED) reporting an attempted suicide by cutting the left forearm veins and ingesting approximately 200 mL of an herbicide (Myrtos®, containing 36% of glyphosate as isopropylamine salt). Laboratory tests showed metabolic acidosis. Hydration with normal saline and alkalinization with sodium bicarbonate was started according to suggestion of the poison control center, since an antidote was unavailable. Cardiorespiratory condition gradually worsened, so that non-invasive positive pressure ventilation (NIPPV) was applied and infusion of fluids was established. Nevertheless, the patient deteriorated and he needed to be transferred to the Intensive Care Unit (ICU), where he underwent orotracheal intubation and invasive mechanical ventilation. Noradrenaline and adrenaline

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Reference	Evaluation by applicant	Abstract
		were infused and fluid resuscitation with crystalloids was incremented. An esophagogastroduodenoscopy (EGD) showed diffuse mucosal erosions of upper digestive tract. No signs of visceral perforation were found during ICU stay. In the following days, the clinical conditions improved and a new EGD showed marked improvement of erosive lesions. After 12 days of ICU stay, the patient was extubated and then transferred to the Psychiatric Unit, in good clinical conditions. Gliphosate ingestion is associated with rapid development of multiple organ failure (MOF). Since an effective antidote is unavailable, major attention should be placed to aggressive life-support care and careful monitoring of complications.
Vidyadhara et al., 2014 Indian Journal of Critical Care Medicine (2014), Vol. 18, Suppl. 1, pp. S36.	This is a report about multiorgan failure after suicidal ingestion of formulated glyphosate and should not impact re-registration.	A 55 years old man self-presented to our Emergency Department (ED) reporting an attempted suicide by cutting the left forearm veins and ingesting approximately 200 mL of an herbicide (Myrtos®, containing 36% of glyphosate as isopropylamine salt). Laboratory tests showed metabolic acidosis. Hydration with normal saline and alkalinization with sodium bicarbonate was started according to suggestion of the poison control center, since an antidote was unavailable. Cardiorespiratory condition gradually worsened, so that non-invasive positive pressure ventilation (NIPPV) was applied and infusion of fluids was established. Nevertheless, the patient deteriorated and he needed to be transferred to the Intensive Care Unit (ICU), where he underwent orotracheal intubation and invasive mechanical ventilation. Noradrenaline and adrenaline were infused and fluid resuscitation with crystalloids was incremented. An esophagogastroduodenoscopy (EGD) showed diffuse mucosal erosions of upper digestive tract. No signs of visceral perforation were found during ICU stay. In the following days, the clinical conditions improved and a new EGD showed marked improvement of erosive lesions. After 12 days of ICU stay, the patient was extubated and then transferred to the Psychiatric Unit, in good clinical conditions. Gliphosate ingestion is associated with rapid development of multiple organ failure (MOF). Since an effective antidote is unavailable, major attention should be placed to aggressive life-support care and careful monitoring of complications.

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Reference	Evaluation by applicant	Abstract
Wang D. et al., 2019 Medicine (2019), Vol. 98, No. 6., pp. e14414	This article describes using ECMO to manage a patient with multiorgan failure after formulated glyphosate and diquat ingestion. Since this is describing medical management of suicidal overdoses, it should not impact re-registration	This article requires a subscription to view the full text. If you have a subscription you may use the login form below to view the article. Access to this article can also be purchased. A 66-year-old man, with a history of Wolff-Parkinson-White syndrome, coronary artery disease, left partial colectomy for polyps, and alcohol abuse (50 g/d), attempted suicide by ingestion of commercially available glyphosate (Round-Up, 200 mL). He was admitted to the emergency ward for monitoring and hyperhydration. First clinical and biological workup and gastric endoscopic examinations were normal.
Wu M-H. et al., 2015 Clinical Toxicology (2015), Vol. 53, No. 4, pp. 330	This is a report about renal failure and haemodialysis after suicidal ingestion of formulated glyphosate and should not impact re-registration.	INTRODUCTION: The mechanisms underlying early central nervous system (CNS) signs and symptoms of glyphosate-surfactant herbicide (GlySH) poisoning are unclear. CASE PRESENTATION: A 58-year-old woman ingested approximately 150 mL of GlySH containing 41% glyphosate and 15% polyoxyethyleneamine. Two days later, she was admitted in the Emergency Center in a semicomatose state. Acute respiratory distress syndrome, circulatory collapse, acute renal failure, and disseminated intravascular coagulopathy were diagnosed. Meningitis was also suspected as she demonstrated Kernig's sign and significant neck stiffness with rigidity of the extremities as well as consciousness disturbance and fever (38.4°C). Investigations of cerebrospinal fluid (CSF) revealed the presence of glyphosate (122.5 μg/mL), significant elevation of IL-6 (394 μg/mL), and pleocytosis (32 cells/μL) with monocyte dominance. All bacteriological and virological tests were later found to be negative. She recovered completely after responding to aggressive supportive care in the intensive care unit. All signs and symptoms suggesting meningitis resolved as the concentration of glyphosate in CSF decreased. She was discharged on day 39 of hospitalization. DISCUSSION: These findings suggest that the present case involved aseptic meningitis in association with GlySH poisoning. CONCLUSION: CNS signs and symptoms induced by aseptic meningitis should be considered in cases of glyphosate-surfactant herbicide poisoning.
You Y. et al., 2012 The American journal of emergency medicine (2012), Vol. 30, No. 9, pp.	This article is discussing the efficacy of intravenous fat emulsion as therapy for formulated glyphosate overdose. This report contributes to the evidence that intravenous fat emulsion may be a useful treatment for glyphosate	Glyphosate herbicide is promoted by the manufacturer as having no risks to human health, with acute toxicity being very low in normal use. In Thailand, however, poisoning from glyphosate agricultural herbicides has been increasing. A case of rapid lethal intoxication from glyphosate-surfactant herbicide involved

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Reference	Evaluation by applicant	Abstract
2097.e1	overdose as it may limit the toxicity associated with large surfactant ingestions. There are no RCTs for this as it is a suicidal overdose situation.	a 37-year-old woman, who deliberately ingested approximately 500 mL of concentrated Roundup formulation (41% glyphosate as the isopropylamine salt and 15% polyoxyethylene amine; Mosanto Company). The postmortem examination revealed that the stomach contained 550 mL of yellow fluid. The gastric mucosa of anterior fundus revealed hemorrhage and the small intestines had marked dilatation and thin walls. We used the high-performance liquid chromatography method for determination of serum and gastric content levels of glyphosate. The glyphosate levels of serum and gastric content were 3.05 and 59.72 mg/mL, respectively. Toxic effects of polyoxyethylene amine and Roundup were caused by their ability to erode tissues including mucous membranes and linings of the gastrointestinal and respiratory tracts. A mild degree of pulmonary congestion and edema was observed in both lungs. We proposed that the characteristic picture of microvesicular steatosis of the hepatocytes, seen predominantly in centrilobular zones of the liver, resembled drug-induced hepatic toxicity or secondary hypoxic stress.
Yu G. C. et al., 2017 Chinese journal of industrial hygiene and occupational diseases (2017), Vol. 35, No. 5, pp. 382	This is a case study describing the clinical course of 10 patients who drank formulated glyphosate. There were no long-term sequelae of ingestion, and all 10 patients survived. These were suicidal ingestions and should not impact re-registration.	The literature, particularly from India, is scarce on the renal effects of glyphosate poisoning. Glyphosate causes toxicity not only after its ingestion but also after dermal exposure by inhalation route and on eye exposure. We present a patient report of glyphosate consumption which resulted in toxic epidermal necrolysis - the first report after glyphosate consumption and acute kidney injury.
Zouaoui K. et al., 2013 Forensic science international (2013), Vol. 226, No. 1-3, pp. E20	This report demonstrates a link between higher blood and urine concentrations with formulated glyphosate overdoses and a poorer outcome. This is unsurprising as it reflects that patients drank a larger volume. Larger volumes of formulated product are associated with more toxicity due to the caustic nature of the surfactant, not the amount of active ingredient. All of the laboratory parameters are expected in critically ill patients. As these were suicidal ingestions, this paper should not impact re-registration.	An 86-year-old woman intentionally drank approximately 300 mL of a glyphosate-surfactant. She was found with consciousness disturbance and experienced several vomiting episodes. On arrival, serum biochemistry revealed a decreased level of butyrylcholinesterase (B-CHE) [11 (normal range: 180-450) IU/L]. Later, her B-CHE level further decreased to single-digit values, and she became comatose with involuntary movement and an increase in muscle tone. Her consciousness level and muscle tone improved with the recovery of her B-CHE level. Physicians should be alert for the occurrence of intermediate syndrome when the B-CHE levels of patients who have consumed a massive amount of glyphosate-surfactant show a prolonged decrease.

Appendix to B.6.9 Overview of publications related to medical data and information that are classified by the applicant as "non-relevant after detailed assessment of full-text article"

To complement the standard toxicity studies, the applicant has performed a literature search in accordance with the EFSA Guidance document EFSA Journal 2011;9(2):2092 "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009". The results were categorized as "non-relevant", as "potentially relevant" or to be of "unclear relevance" following a rapid assessment. For the two latter categories, the full-text documents were reviewed in detail and then categorized as "non-relevant" or "relevant". The articles considered relevant were categorized as A (providing data for establishing or refining risk assessment parameters), as B (articles relevant to the data requirement but in opinion of the applicant only to provide supplementary information that does not alter existing risk assessment) or as C (articles of unclear relevance).

More details on the literature search, including tables describing the studies in categories A, B and C can be found in Volume 3 CA section B.6.10.1.

For several studies related to medical data and information that were classified by the applicant as "non-relevant after detailed assessment of full-text articles", the AGG requested study summaries to further justify the categorization of the information. The justification provided by the applicant was reviewed by the RMS and the assessments are presented in this appendix for these studies.

Overview of studies categorised by the applicant as non-relevant[15] after rapid assessment of title/abstract for which the RMS requested a summary in order to further justify the categorization.

No.	Technical section	Author	Year	Title
10521	Toxicology and metabolism	Andreotti, Gabriella (correspondence); Hou, Lifang; Beane Freeman, Laura E.; Mahajan, Rajeev; Koutros, Stella; Coble, Joseph; Lubin, Jay; Blair, Aaron; Alavanja, Michael	2010	Body mass index, agricultural pesticide use, and cancer incidence in the Agricultural Health Study cohort.
10811	Toxicology and metabolism	Hohenadel, Karin (correspondence); Harris, Shelley A.; Demers, Paul A.; Blair, Aaron	2011	Exposure to multiple pesticides and risk of non-Hodgkin lymphoma in men from six Canadian provinces.
11019	Toxicology and metabolism	Nevision Cynthia D	2014	A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors.

Data point	CA 9
Author	Andreotti G. *et al.*
Year	2010
Title	Body mass index, agricultural pesticide use, and cancer incidence in the

[15] The applicant has provided an Excelsheet in which all articles are presented that are considered non-relevant after rapid assessment (based on title/abstract). These were checked by the RMS and for the articles listed in the table the RMS has requested a summary in order to further justify the categorization of the study.

	Agricultural Health Study cohort
Document source	Cancer Causes and Control, (2010) Vol. 21, No. 11, pp. 1759-1775
Short description of literature article	The investigators sought to evaluate the interaction between obesity and pesticide use on cancer risk among participants in the Agricultural Health Study (AHS). They first evaluated whether 17 cancers were associated with obesity (body mass index [BMI]) among AHS men or women who had self-reported height and weight data. They then focused on statistical interactions for 22 pesticides and on trends in cancer risk with increasing BMI for non-users and users of these 22 pesticides. This was largely a hypothesis generating study. As the authors noted, "since the mechanisms by which pesticides may interact with BMI on cancer risk in humans are unclear, our results should be considered hypothesis generating" (p 1773).
Short description of findings	BMI was positively associated with colon cancer in men and breast cancer in postmenopausal women. In contrast, BMI was inversely associated with lung cancer in men, with a significant association in ever smokers and a null association in never smokers. Interaction between BMI and colon cancer in men was significant in those who ever used carbofuran (HR = 1.10, 95% CI 1.04–1.17; p-interaction = 0.04) or metolachlor (HR = 1.09, 95% CI 1.04–1.15; p-interaction = 0.02) but not among users of other pesticides (including glyphosate). Among male ever smokers, the inverse association between BMI and lung cancer was significant in non-users of carbofuran (HR = 0.87, 95% CI = 0.82–0.92) but was null in users of carbofuran (p-interaction = 0.02). Most pesticides, glyphosate included, showed trends of increasing risk of colon cancer with increasing BMI among users, but not among non-users. The authors concluded that their findings suggest that certain pesticides may modify the risk of colon and lung cancer associated with obesity.
Justification as provided in the AIR5 dossier (KCA 9)	Not relevant by title/abstract: This is a conference abstract only. Observations are caused by mixture of compounds/potentially causal factors and thus not attributable to a substance of concern. **Further points for clarification:** This study has no relevance to the ongoing evaluation of glyphosate. It is uncertain what to make of results based on a speculative hypothesis about pesticides and obesity. Glyphosate did not show a statistical interaction for obesity (BMI) and colon cancer and it was among many pesticides for which colon cancer risk increased with increasing BMI among ever users. None of the analyses of individual pesticides was controlled for the effects of other pesticides and the extent of the various pesticide exposures was not considered, so the interpretation of results for any individual pesticide is uncertain. Results from the latest glyphosate AHS publication show no relationship between ever-using glyphosate and colon cancer risk and no relationship between the extent of glyphosate exposure and colon cancer risk (see Table 1). Table 1. Colon cancer findings by glyphosate exposure level in the Agricultural Health Study (Andreotti G. *et al*., 2018)

Exposure quintile	# cases	Relative Risk	95% CI	P value for trend
None	116	1.0	reference	
Q1	104	1.0	0.7-1.4	
Q2	102	1.0	0.8-1.4	

Q3	102	1.1	0.8-1.4	
Q4	96	1.0	0.7-1.4	1.0

In this latest AHS publication, the authors make no mention of obesity as a modifying factor, though the first author is the same for both papers. One can only assume that this 2010 publication was hypothesis generating and did not pan out as promising for subsequent AHS research for glyphosate or other pesticides.

References

Andreotti G, Koutros S, Hofmann JN, *et al*. Glyphosate use and cancer incidence in the Agricultural Health Study. Journal of the National Cancer Institute, (2018) Vol. 110, No. 5, pp. 509-516

RMS comments

The RMS does agree with the applicant's justification that the study has no relevance to the assessment on glyphosate. It is noted, however, that the publication is not a conference abstract but a peer-reviewed open-literature publication.

Data point	CA 9
Author	Hohenadel K. *et al*.
Year	2011
Title	Exposure to multiple pesticides and risk of non-Hodgkin lymphoma in men from six Canadian provinces
Document No	International Journal of Environmental Research and Public Health (2011), Vol. 8, No. 6, pp. 2320-2330
Short description of literature article	The authors analysed data from a six-province Canadian case-control study that was conducted between 1991 and 1994 to investigate the relationship between non-Hodgkin's lymphoma (NHL), the total number of pesticides used, and some common pesticide combinations. Cases (n = 513) were identified through hospital records and provincial cancer registries and controls (n = 1506) were frequency matched to cases by age and province of residence. Logistic regression analysis was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs).
Short description of findings	The authors concluded that risk of NHL increased with the number of pesticides used. ORs increased further when only "potentially carcinogenic" pesticides[16] were considered (OR[one pesticide] = 1.30, 95% CI = 0.90–1.88; OR[two to four] = 1.54, 95% CI = 1.11–2.12; OR[five or more] = 1.94, 95% CI = 1.17–3.23). Elevated risks were also found among those reporting use of malathion in combination with several other pesticides. These analyses were considered supportive of the hypothesis that the risk of NHL increases with the number of pesticides used and some pesticide combinations.
Justification as provided in the AIR5 dossier (KCA 9)	Not relevant by title/abstract: Observations is caused by mixture of compounds/ potentially causal factors and thus not attributable to a substance of concern. Further points for clarification: This paper has limited relevance for the ongoing evaluation of glyphosate.

[16] Pesticides considered potentially carcinogenic in this analysis did not include glyphosate and were: 2,4,5-T, 2,4-D, 2,4-DB, arsenic, asulam, benomyl, bromoxynil, carbaryl, cypermethrin, DDT, dicamba, diclofop-methyl, dieldrin, dimethoate, dinoseb, formaldehyde, heptachlor, lindane, linuron, mancozeb, MCPA, mecoprop, methidathion, paraquat, propoxur, toxaphene, triallate, trichloroacetic acid, trifluralin.

The purpose of this study was to evaluate the hypothesis that exposures to combinations of pesticides might increase the risk of NHL. For this purpose, the authors reanalysed data from the 2001 publication by McDuffie and colleagues (2001). ORs were calculated individually and jointly for 36 combinations of pesticides, including 5 combinations for glyphosate: bromoxynil/glyphosate, carbathin/glyphosate, 2,4-D/glyphosate, malathion/glyphosate, and mecoprop/glyphosate. The general scheme for the joint effect analyses was to calculate an interaction contrast ratio ($ICR = OR_{both\ pesticides} - OR_{pesticide\ 1\ only} - OR_{pesticide\ 2\ only} + 1$). An additive effect of the combination exposure was pre-specified as an ICR >0.50.

Focusing on glyphosate, results for malathion and glyphosate were detailed in the article (see table). Results for the other glyphosate combinations were not reported. The OR for malathion alone was appreciably elevated. The OR for glyphosate was essentially null. In combination, the OR was essentially the same as it was for malathion alone. The ICR was well below the value pre-specified to indicate appreciable additivity and the related interaction p value was 0.64. As such, these analyses show no indication of interaction with glyphosate. Give that the authors did not present the results for the other 4 glyphosate combinations, one can presumably conclude that there was no evidence of interaction in those analyses as well.

Chemical	OR	95% CI	ICR	P value
Malathion	1.95	1.29-2.93		
Glyphosate	0.92	0.54-1.55		
Malathion & glyphosate	2.10	1.31-3.37		
Interaction			0.19	0.64

There are a lot of limitations of this study. Some have been discussed previously in the context of the McDuffie publication. Most prominent is the issue of recall bias. Crump pointed out that 93% of the ORs for individual pesticides in this study were > 1.0, when, assuming most pesticides are not carcinogenic for NHL, one would expect a distribution of ORs above and below 1.0 (Crump 2020). Crump attributed the skewed positive nature of the findings to be due to a combination of recall and selection bias. Hence, any re-analyses of the data from McDuffie *et al*. (2001) are likely to be biased toward positive values.

A unique limitation to this reanalysis is the uncertainty about what combination exposure means. So-called combination exposures could have been from tank mixtures (viz., true combination exposure), applications at different times during the year, or applications years apart. So, the exposure metric used in this study is poorly suited for the study of combination exposures. Nonetheless, there were no indications in this study of a carcinogenic effect of glyphosate either alone or in combination with 5 other pesticides.

References

Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Anal 2020; 40(4): 696-704.

McDuffie HH, Pahwa P, McLaughlin JR, *et al*. Non-Hodgkin's lymphoma and specific pesticide exposures in men: Cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev 2001; 10:1155-1163.

RMS comments

Based on the abstract and the justification provided by the applicant, the RMS does agree that the study is not relevant for the current assessment. The original analysis of the data is already discussed in the dossier (McDuffie et al. (2001,

refer to Vol 3 CA B.6.5.18.23)). There is no added value of this re-analysis investigating combinations of pesticide use (in this case malathion and glyphosate) as it is not described how the so-called combination exposure is defined. As already addressed by the applicant, this could be use of tank mixtures (thus a true combination exposure), applications at different times during the year, or applications years apart. Besides the issue of recall bias as described by the applicant, it is further noted that no adjustment for confounders were made in the analysis (except for age and province of residence).

Data point	CA 9
Author	Nevison C. D.
Year	2014
Title	A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors
Document source	Environmental Health (2014), Vol. 13, pp. 73
Short description of literature article	The author evaluated trends in autism prevalence from 1970 to 2005 trying to factor out increased diagnosis. She then compared the trend line for "corrected" autism prevalence with those for a list of toxins that are increasing in use, including glyphosate.
Short description of findings	The author concluded that approximately 80% of the increase in autism is real. She also concluded that toxins with increasing trends in the environment can suggest hypotheses for causal factors for autism.
Justification as provided in the AIR5 dossier (KCA 9)	Not relevant by title/abstract: Trend analysis of autism and various factors including glyphosate. Further points for clarification: This article is not relevant to the ongoing evaluation of glyphosate. The author does not actually link any of the environmental toxins to autism patients on an individual level as might be done in an epidemiologic study. There is no data presented to indicate that any autism patient over the study period had exposure to glyphosate or the other listed toxins. The paper merely speculates that two things increasing over the same time period might be related. Many things have increased over the 1970-2005 time period, some that children are much more likely to encounter than glyphosate or the other environmental exposures considered in this paper (e.g., organic foods, cell phones, PCs, etc.). So, this research is ill-founded in terms of advancing knowledge about likely causes of autism and does not provide any relevant information about glyphosate.
RMS comments	The RMS does agree with the applicant that the study has no relevance to the assessment on glyphosate as the study does not investigate a direct link between actual exposure to glyphosate and the occurrence of autism. However, the RMS disagrees with the applicant's statement that the research was "ill-funded in terms of advancing knowledge about likely causes of autism" since it is explicitly stated in the publication that the present study is not proof of causation. The author is of the opinion that considering timing of first diagnosis and upsurge of cases of autism in the 1980's did not coincide with the widespread use of glyphosate in the US. The author speculates about the interacting effects of glyphosate and other toxins however.

B.6.10. REFERENCES RELIED ON

B.6.10.1. Literature search

Initial literature search

A literature search for glyphosate and its metabolites (AMPA, n-acetyl-AMPA, N-acetyl-glyphosate, HMPA, n-methyl-AMPA, N-glyceryl-AMPA, n-malonyl-AMPA, methylphosphonic acid and n-methylglyphosate) was conducted in accordance with the EFSA Guidance document (EFSA Journal 2011;9(2): 2092).

Some recommendation were made by the AGG on how to present the literature search in the dossier which have been followed. These recommendation included that the GRG submitted an Excel sheet of all studies excluded based on the rapid assessment including a justification on a general level. This Excel sheet has been checked by the AGG and based on this assessment some additional public literature studies were requested.

The objective of the study was to identify all relevant literature from the last 10 years.

The GRG identified 60 studies that they identified as relevant studies, 265 that they identified as relevant but supplementary, 6 that they identified as articles of unclear relevance and 292 were identified as non-relevant.

Top-up literature search 2020

An additional literature search was performed by GRG covering the publication period of January 2020 to June 2020, as requested by the AGG.

The GRG identified 7 studies that they identified as relevant studies, 18 studies that they identified as relevant but supplementary and 71 studies were identified as non-relevant.

2. Search strategy

2.1 Data of the search

Due to the sheer volume of available literature, the search was divided in six parts. An overview is included in the Table below.

Search	Performed for	Covering publication period	Conducted on
Part 0	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jan 2010 – Dec 2011	28th Oct 2019
Part 1	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jan 2012 – Dec 2017	08th Jun 2018.
Part 2a	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jan 2018 – Dec 2018	04th Jul 2019
Part 2b		Jan 2019 – Jun 2019	10th Jul 2019
Part 3	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jul 2019 – Dec 2019	7th Jan 2020
Part 4	HMPA	Jan 2010 – Feb 2020	24th Feb 2020
Part 5a	N-methyl-AMPA, N-glyceryl-AMPA, N-malonyl-AMPA	Jan 2010 – Feb 2020	27th Feb 2020
Part 5b	methylphosphonic acid	Jan 2010 – Feb 2020	27th Feb 2020
Part 6	N-methylglyphosate	Jan 2010 – April 2020	04th May 2020

AMPA = (aminomethyl)phosphonic acid
HMPA = (hydroxymethyl)phosphonic acid

Top-up literature search 2020
The top-up search and evaluation of glyphosate public literature covers the publication period of January 2020 to June 2020. The month of December 2019 was already comprehensively covered by the previously submitted Literature Review.

Performed for	Covering publication period	Conducted on
Glyphosate AMPA N-acetyl-AMPA N-acetyl-glyphosate HMPA N-methyl-AMPA N-glyceryl-AMPA N-malonyl-AMPA methylphosphonic acid N-methylglyphosate	January 2020 – June 2020 (incl. June 2020)	02-July 2020

AMPA = (aminomethyl)phosphonic acid
HMPA = (hydroxymethyl)phosphonic acid

2.2 Time window of the literature search

See 2.1.

2.3 Bibliographic Databases used in the literature review

The bibliographic databases used were: Agricola, BIOSIS, CABA, CAPLUS, MEDLINE, EMBASE, TOXCENTER, FSTA, PQSCITECH, ESBIOBASE, SCISEARCH.

For the 2020 top-up search and evaluation of glyphosate public literature the same databases were used.

The databases used are considered to be acceptable.

2.4 Input parameters for literature search:

Input parameters glyphosate

Substance name	Glyphosate Salts: isopropylamine, potassium, ammonium, methylmethanamine
IUPAC/CA name	2-(phosphonomethylamino)acetic acid
CAS number(s)	1071-83-6 Salts: 38641-94-0, 70901-12-1, 39600-42-5, 69200-57-3, 34494-04-7, 114370-14-8, 40465-66-5, 69254-40-6

Input parameters metabolite metabolites

AMPA	
Substance name	AMPA
IUPAC/CA name	(aminomethyl)phosphonic acid
CAS number(s)	1066-51-9
N-acetyl glyphosate	
Substance name	N-acetyl glyphosate
IUPAC/CA name	N-acetyl-N-(phosphonomethyl)glycine
CAS number(s)	129660-96-4

N-acetyl AMPA	
Substance name	N-acetyl AMPA
IUPAC/CA name	[(acetylamino)methyl]phosphonic acid
CAS number(s)	57637-97-5
HMPA	
Substance name	HMPA
IUPAC/CA name	(hydroxymethyl)phosphonic acid
CAS number(s)	2617-47-2
N-methyl AMPA	
Substance name	N-methyl AMPA
IUPAC/CA name	[(methylamino)methyl]phosphonic acid
CAS number(s)	35404-71-8
N-glyceryl AMPA	
Substance name	N-glyceryl AMPA
IUPAC/CA name	(2,3-dihydroxypropanoylamino)methylphosphonic acid
CAS number(s)	-
N-malonyl AMPA	
Substance name	N-malonyl AMPA
IUPAC/CA name	3-oxo-2-(phosphonomethylamino)propanoic acid
CAS number(s)	-
Methylphosphonic acid	
Substance name	Methylphosphonic acid
IUPAC/CA name	Methylphosphonic acid
CAS number(s)	993-135
N-methyl glyphosate	
Substance name	N-methyl glyphosate
IUPAC/CA name	2-[methyl(phosphonomethyl)amino]acetic acid
CAS number(s)	24569-83-3

2.5 Endpoint specific search terms

As the number of records returned by the single search concept was extremely large a focused search was conducted using the search terms as indicated in the table below.

Table 16: Keywords used for the active substance glyphosate and its metabolites

Gly1: Glyphosate and AMPA	glyphosat? OR glifosat? OR glyfosat? OR 1071-83-6 OR 38641-94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR 40465-66-5 OR 69254-40-6 OR aminomethyl phosphonic OR aminomethylphosphonic OR 1066-51-9
Gly2: N-acetyl glyphosate and N-acetyl AMPA	2 acetyl phosphonomethyl amino acetic acid OR n acetyl glyphosate OR n acetylglyphosate OR n acetyl n phosphonomethyl glycine OR 129660-96-4 OR n acetyl ampa OR acetylamino methyl phosphonic acid OR acetylaminomethyl phosphonic acid OR 57637-97-5
HMPA	2617-47-2 OR hydroxymethanephosphonic acid OR hydroxymethyl phosphonate OR hydroxymethylphosphonate OR hydroxymethyl phosphonic acid OR hydroxymethylphosphonic acid OR methanehydroxyphosphonic acid OR phosphonic acid(1w)hydroxymethyl OR phosphonomethanol
N-methyl AMPA	35404-71-8 OR methylamino methyl phosphonic acid OR methylaminomethyl phosphonic acid OR methylaminomethylphosphonic acid OR n methyl ampa OR nsc 244826 OR phosphonic acid methylamino methyl OR phosphonic acid p methylamino methyl
N-glyceryl AMPA	2 3 dihydroxy 1 oxopropyl aminomethyl phosphonic acid OR 2 3 dihydroxy 1 oxopropyl aminomethylphosphonic acid OR n glyceryl ampa
N-malonyl AMPA	3 oxo 3 phosphonomethyl amino propanoic acid or 3 oxo 3 phosphonomethyl aminopropanoic acid or n malonyl ampa
methylphosphonic acid	993-13-5 OR dihydrogen methylphosphonate OR methanephosphonic acid OR methyl phosphonic acid OR methylphosphonic acid OR nsc 119358 OR phosphonic acid methyl OR phosphonic acid p methyl
N-methylglyphosate (NMG)	24569-83-3 OR 2 methyl phosphonomethyl amino acetic acid OR 2 methyl phosphonomethyl aminoacetic acid OR acetic acid 2 n methyl n phosphonatomethyl amino OR glycine n methyl n phosphonomethyl OR glyphosate n methyl OR methyl glyphosate OR methyl phosphonomethyl amino acetic acid OR methyl phosphonomethyl aminoacetic acid OR n methyl n phosphonomethyl glycine OR n methylglyphosate OR n phosphonomethyl n methyl glycine OR n phosphonomethyl n methylglycine

(1w) = proximity operator (this order, up to 1 word between)

Table 17: Search filters related to the technical section toxicology

Toxicology Gly1 OR Gly2 AND the following search filters; methyl phosphonic acid AND the following search filters
tox? OR hazard? OR adverse OR health OR NOAEL OR NOEL OR LOAEL OR LOEL OR BMD? OR in vivo OR in vitro OR invivo OR invitro OR mode of action OR skin? OR eye? OR irrit? OR sensi? OR allerg? OR rat OR rats OR dog? OR rabbit? OR guinea pig? OR mouse OR mice OR metabolism OR metabolite? OR metabolic OR distribution OR adsorption OR excretion OR elimination OR kinetic OR cytochrome OR enzym? OR gen? OR muta? OR chromos? OR clastogen? OR DNA OR carcino? OR cancer? OR tumor? OR tumour? OR oncog? OR oncol? OR malign? OR immun? OR neur? OR endocrin? OR hormon? OR gonad? OR disrupt? OR reproduct? OR development? OR malform? OR anomal? OR fertil? OR foet? OR fet? OR matern? OR pregnan? OR embryo? OR epidem? OR medical? OR poison? OR exposure OR operator? OR bystander? OR resident? OR worker? OR occupat? biomonitoring OR human exposure OR microbiome OR oxidative stress OR apoptosis OR necrosis OR cytotoxicity OR Polyoxyethyleneamine OR POEA OR surfactant OR risk assessment?

The search terms used are in line with the examples given in the Appendix to the EFSA guidance on the conduct of a literature search. ***However, the RMS notes that the search terms used are focussed on the data requirements and some specific search term which are considered relevant for human health are missing. For example, a quick search by the RMS retrieved the following publications which were not found in the literature search by the applicant:***

1) Rueda-Ruzafa, L., Cruz, F., Roman, P., Cardona, D. Gut microbiota and neurological effects of glyphosate. (2019) NeuroToxicology
2) Pu Y, Yang J, Chang L, Qu Y, Wang S, Zhang K, Xiong Z, Zhang J, Tan Y, Wang X, Fujita Y, Ishima T, Wang D, Hwang SH, Hammock BD, Hashimoto K. Maternal glyphosate exposure causes autism-like behaviors in offspring through increased expression of soluble epoxide hydrolase. Proc Natl Acad Sci U S A. 2020 May 26;117(21):11753-11759.

The applicant is requested to provide an additional literature search using endpoint specific search terms related to human health which are outside the data requirements such as autism, asthma, ADHD, coeliac disease, inflammatory bowel disease and obesity. The applicant is requested to submit all relevant publications obtained from this search including a summary and an evaluation of these publications (including a relevance and reliability assessment).

Top-up literature search 2020

The same input parameters for literature search, endpoint specific search terms and filters were used for the 2020 top-up search, leading to the following search results:

Total number of hits from all search:	2156
Total number of hits after removal of duplicates*:	852

* duplicates within the current search and entries found already in the previous search.

2.6 Filters
Endpoint specific search filters were applied as described in section 2.5.

3. Search results

Original search (results by database)

AGRICOLA:	Part 0: 412; Part 1: 1483; Part 2: 494; Part 3: 181; Part 4: 4; Part 5a&b: 0&91; Part 6: 6
BIOSIS:	Part 0: 583; Part 1: 2216; Part 2: 792; Part 3: 224; Part 4: 10; Part 5a&b: 1&150; Part 6: 6
CABA:	Part 0: 1018; Part 1: 3418; Part 2: 669; Part 3: 377; Part 4: 3; Part 5a&b: 0&36; Part 6: 16
CAPLUS:	Part 0: 899; Part 1: 3036; Part 2: 809; Part 3: 339; Part 4: 28; Part 5a&b: 4&616; Part 6: 27
MEDLINE:	Part 0: 249; Part 1: 1188; Part 2: 573; Part 3: 185; Part 4: 12; Part 5a&b: 1&198; Part 6: 7
EMBASE:	Part 0: 335; Part 1: 1390; Part 2: 628; Part 3: 159; Part 4: 22; Part 5a&b: 1&426; Part 6: 7
TOXCENTER:	Part 0: 738; Part 1: 2935; Part 2: 993; Part 3: 381; Part 4: 19; Part 5a&b: 4&353; Part 6: 19
FSTA:	Part 0: 33; Part 1: 176; Part 2: 52; Part 3: 27; Part 4: 1; Part 5a&b: 0&2; Part 6: 2
PQSCITECH:	Part 0: 468; Part 1: 1043; Part 2: 169; Part 3: 100; Part 4: 3; Part 5a&b: 0&72; Part 6: 6
ESBIOBASE:	Part 0: 390; Part 1: 1421; Part 2: 566; Part 3: 163; Part 4: 10; Part 5a&b: 1&58; Part 6: 8
SCISEARCH:	Part 0: 815; Part 1: 3236; Part 2: 1155; Part 3: 370; Part 4: 22; Part 5a&b: 1&329; Part 6: 12

Total number of hits from all search:	39482
Total number of hits after removal of duplicates:	13707
Total number of hits after merge of all sections:	11326
Total number of hits for toxicology section:	1454

Top-up literature search 2020

For the 2020 top-up search the same databases were used, with the same details of the search except that the date of search is 2 July 2020 and the latest database update all took place in June or July of 2020. The total number of records retrieved per database were as follows:

AGRICOLA:	258
BIOSIS:	321
CABA:	467
CAPLUS:	364
MEDLINE:	207
EMBASE:	166
TOXCENTER:	470
FSTA:	20
PQSCITECH:	106
ESBIOBASE:	206
SCISEARCH:	571

Total number of hits from all search:	2156
Total number of hits after removal of duplicates*:	852
Total number of hits for toxicology section:	96

* duplicates within the current search and entries found already in the previous search.

The databases used by GRG for the literature search are considered acceptable.

4. Evaluation

4.1 Rapid assessment

For the rapid assessment articles were evaluated at title/abstract level.

Articles were identified as non-relevant in the rapid assessment based on the following criteria:

- Publications related to efficacy
- Publications dealing with analytical methods/development
- Publications describing new methods of synthesis or other aspects of basic chemistry
- Patents
- Wastewater treatment
- Abstracts referring to a conference contribution that does not contain sufficient data
- Publications focusing on genetically modified organisms/transgenic crops
- Publications where glyphosate or a relevant metabolite were not the focus of the paper
- Secondary information includes scientific and regulatory reviews
- Articles dealing with political/socio/economic analysis
- Observations caused by mixture of compounds/potentially causal factors and thus not attributable to a substance of concern (e.g. mixture toxicity)
- Study design, test system, species tested, exposure route etc are not relevant for the European regulatory purposes
- Publications not dealing with EU representative uses/conditions

On request of the AGG the GRG supplied an Excel list of all studies excluded based on the rapid assessment. This list was checked by the AGG and three additional studies were requested, provided by GRG and included in the dossier. These mainly related to studies which the applicant considered to only evaluate mixture effect while a detailed check of the study report showed that individual compounds analysis were also included.

The same rapid assessment was performed for the top-up literature search 2020.

4.2 Detailed assessment

For the detailed assessment full-text documents have been reviewed.

Articles that have been identified as non-relevant in the detailed assessment belong to one of the following categories:

- Publications dealing with a Roundup formulation that is not the representative formulation for the AIR5 renewal in Europe.
- Publications dealing with general pesticide exposure.
- The presented endpoints are not relatable to the EU level risk assessment
- Opinion articles where no new data is provided that can be used for risk assessment
- Findings based on cellular and molecular level that cannot be related to risk assessment
- Criteria outlined in section 4.1 that needed the full text document to determine.

Some specific criteria were applied for articles on human health. In case of *in vitro* toxicity tests studies that tested beyond 1 mM were not considered to be relevant. The reason for this is because it is physiologically not possible to attain such concentrations in regulatory *in vivo* testing due to the limited oral bioavailability (appr. 20%), low dermal absorption and rapid excretion. Further justification on the selection of the 1 mM limit can be found in doc K-CA section 9.

The applicant provided the following justification in KCA-9 (report no 108689-CA9-1, 2020):
" *The limit of 1 mM has been based on the single dose oral pharmacokinetic data of a formulation containing 71.7% w/w glyphosate where an oral dose of 1,430 mg/kg bw in the rat gives plasma levels of 38.1 µg/mL or 0.225 mM after 2 hours. When extrapolated linearly (which is possible for glyphosate because it is not subject to hepatic metabolism) this gives plasma levels of 53.3 µg/mL or 0.315 mM at 2 hours after oral intake of 2,000 mg/kg bw and 107 µg/mL or 0.630 mM at 2 hours after oral intake of 4,000 mg/kg bw. A systemic concentration of glyphosate of 1 mM would then represent an oral dose of more than 6,000 mg/kg bw which is completely unreasonable for repeat dose experimental in vivo testing under today's OECD test guidelines. The ADI for glyphosate of 0.5 mg/kg bw/day corresponds with a daily systemic concentration of 0.17 µg/mL or 1 µM when a 60 kg person with 36 L extracellular fluid is considered with a glyphosate oral bioavailability of 20%*."

The RMS largely agrees with the above justification, however, a reference should be given for the study in which an oral dose of 1,430 mg/kg bw (given as a formulation of 71.7% w/w glyphosate) resulted in plasma levels of 38.1 µg/mL in the rat. If the study is not already included in the dossier, the study should be submitted and evaluated. In addition, a further justification should be given on whether locally higher levels of glyphosate at cellular level could be reached (e.g. in intestinal epithelial cells and/or in the local lymphatic vessels of the intestinals).

The applicant divided the studies in 3 categories as recommended in the EFSA GD 2011; 9(2):2092, Point 5.4.1:

- Category (a) Studies that provide data for establishing or refining risk assessment parameters. These studies should be summarised in detail following the subsequent steps of the OECD Guidance documents (OECD, 2005; 2006) and should be considered for reliability.
- Category (b) Studies that are relevant to the data requirement, but in the opinion of the applicant provide only supplementary information that does not alter existing risk assessment parameters. After expert judgement, essential reliability parameters affect the full reliability of the study. A justification for such a decision should be provided.
- Category (c) Studies for which relevance cannot be clearly determined. For each of these studies the applicants should provide an explanation of why the relevance of such studies could not be definitively determined.

	Number	Justification
Total number of records after merge of all searches [a)] and removal of duplicates.	1454	n.a.
Number of articles excluded after rapid assessment (title / abstract).	831	See the Literature Review Excel File.
Total number of full-text documents assessed in detail.	623	n.a.
Number of articles excluded after detailed assessment (*i.e.* not relevant).	292	Table 6.10-4a
Number of articles not excluded after detailed assessment. [b)]	331	See Table 6.10-2a and Table 6.10-3a
Number of summaries presented in the dossier. [c)]	60	See Table 6.10-1a

[a)] After all searches: Part 0, 1, 2, 3, 4, 5a&b, 6.

[b)] All articles belonging to the category A, B, C of the Point 5.4.1 (as stated in the EFSA GD document).

[c)] Summaries presented in the dossier: articles classified as relevant (EFSA GD, Point 5.4.1, category A) & reliable or relevant (EFSA GD, Point 5.4.1, category A) & reliable with restrictions.

The applicant provided a list of all studies excluded based on the detailed assessment. This list was checked by the RMS and additional studies were requested to be submitted which were considered to be potentially relevant. These studies are included in the dossier in the corresponding sections.

Top-up literature search 2020

	Number	Justification
Total number of articles after manual removal of duplicates [a)]	96	n.a.
Number of articles excluded after rapid assessment (title / abstract).	50	See the Literature Review Excel File.
Total number of full-text documents assessed in detail	46	n.a.
Number of articles excluded after detailed assessment (*i.e.* not relevant).	21	Table B.6.10-4b
Number of articles not excluded after detailed assessment [b)]	25	See Table B.6.10-2b and Table B.6.10-3b
Number of summaries presented in the dossier [c)]	7	See Table B.6.10-1b

[a)] After removal of duplicates within the current search (Jan 2020 – Jun 2020) and entries found already in the previous search (Jan 2010 – Dec 2019). Additional duplicates occurred due to different update frequencies within each database and entries of publications ahead of print.

[b)] All relevant articles by full-text belonging to the relevance Category A, B, C (acc. to the EFSA Journal 2011;9(2):2092, Point 5.4.1).

[c)] Summaries were compiled for relevant articles of Category A and classified either as reliable or reliable with restrictions.

In the top-up literature search 2020, a total of 21 articles of the remaining 46 articles were identified as "non-relevant" in the detailed assessment and were excluded from further evaluation. The list of these articles and the justification for their non-relevance is provided and was checked by the RMS.

The remaining 25 articles identified as "relevant" in the detailed assessment were classified according to the EFSA 2092 Guidance Document (EFSA Journal 2011;9(2):2092, Point 5.4.1).

Category A Articles which provide data for establishing or refining risk assessment parameters.
For all articles of Category A, a reliability assessment was performed as recommended in the EFSA 2092 Guidance Document (GD). Summaries were compiled for Category A articles classified as "reliable" or "reliable with restrictions". The list of these Category A & reliable / reliable with restrictions articles can be found below.

Category B Articles relevant to the data requirement but in the opinion of the applicant providing only supplementary information that does not alter existing risk assessment.
A justification for such decision is provided as recommended in the EFSA 2092 Guidance Document (GD). The list of these Category B articles and the justifications is provided and checked by the RMS.

Category C Articles for which relevance cannot be clearly determined.
As recommended in the EFSA 2092 Guidance Document (GD), an explanation is provided why the relevance could not be determined.

The outcome for toxicology according to GRG was that 7 publications were considered Category A, 18 publications were considered Category B (supplementary information) and no publications were considered Category C in the 2020 top-up search.

For both searches, the following criteria have been used by the applicant for reliability assessment

For articles, which have been identified as category A, under the Point 5.4.1 of the EFSA GD document, a reliability assessment has been performed. The reliability criteria for ecotoxicology section are summarized in the table below.
For articles (category A) that have been identified as reliable or reliable with restrictions, summaries have been compiled. Articles of category A which have been identified as non-reliable were downgraded to articles of category B (relevant but supplementary).

Reliability criteria for toxicology – epidemiology and exposure studies

Reliability criteria - toxicology	
Epidemiology studies	**Exposure studies**
Guideline-specific	**Guideline-specific**
Study in accordance to valid internationally accepted testing guidelines/practices	Study in accordance to valid internationally accepted testing guidelines/practices
Study completely described and conducted following scientifically acceptable standards	Study performed according to GLP
	Study completely described and conducted following scientifically acceptable standards
Test substance	**Test substance**
Exposure to formulations with only glyphosate as a.i.	Exposure to formulations with only glyphosate as a.i.
Exposure to formulations with glyphosate combined with other a.i.	Exposure to formulations with glyphosate combined with other a.i.
Exposure to various formulations of pesticides	Exposure to various formulations of pesticides
Study	**Study**
Study design – epidemiological method followed	Study design clearly described
Description of population investigated	Population investigated sufficiently described
Description of exposure circumstances	Exposure circumstances sufficiently described
Description of results	Sampling scheme sufficiently documented
Have confounding factors been considered	Analytical method described in detail

Statistical analysis	Validation of analytical method reported
	Monitoring results reported
Overall assessment: Reliable / Reliable with restrictions / Not reliable	

Reliability criteria for toxicology – *in vitro* and *in vivo* studies

Reliability criteria – toxicology and metabolism	
***In vitro* studies**	***In vivo* studies**
Guideline-specific	**Guideline-specific**
Study in accordance to valid internationally accepted testing guidelines	Study in accordance to valid internationally accepted testing guidelines.
Study performed according to GLP	Study performed according to GLP
Study completely described and conducted following scientifically acceptable standards	Study completely described and conducted following scientifically acceptable standards
Test substance	**Test substance**
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)
Only glyphosate acid or one of its salts is the tested substance	Only glyphosate acid or one of its salts is the tested substance
AMPA is the tested substance	AMPA is the tested substance
Study	**Study**
Test system clearly and completely described	Test species clearly and completely described
Test conditions clearly and completely described	Test conditions clearly and completely described
Metabolic activation system clearly and completely described	Route and mode of administration described
Test concentrations in physiologically acceptable range (< 1 mM)	Dose levels reported
Cytotoxicity tests reported	Number of animals used per dose level reported
Positive and negative controls	Method of analysis described for analysis test media
Complete reporting of effects observed	Validation of the analytical method
Statistical methods described	Analytical verifications of test media
Historical negative and positive control data reported	Complete reporting of effects observed
Dose-effect relationship reported	Statistical methods described
	Historical control data of the laboratory reported
	Dose-effect relationship reported
Overall assessment: Reliable / Reliable with restrictions / Not reliable	

Other considerations by the applicant:

Many articles that have been considered relevant for the risk assessment of glyphosate and have been assessed for reliability on full text basis contain experimental data as well on glyphosate as such as on formulations (different from MON 52276) and co-formulants. In such case only the toxicology data pertinent to glyphosate and to the reference formulation (if that can be clearly stated by the author of the article) are summarized and discussed. In the case of articles on exposure monitoring and epidemiology, exposure to glyphosate formulations are considered.

In the sections below, a list of publications included in the risk assessment (Category A; Tables 6.10-1a and 6.10-1b), a list of studies excluded from the RAR (relevant but providing only supplementary information (Cat B; Tables 6.10-2a and 6.10-2b)) and a list of studies excluded from the RAR (articles for which relevance cannot be clearly determined (Cat C; Tables 6.10-3a and 6.10-3b) and a list of remaining non-relevant studies (Tables 6.10-4a and 6.10-4b) for the initial search and for the 2020 top up search. The tables were provided by the applicant and the RMS has given its comments in the RMS comments column.

Category A

Table B.6.10-1a: Overview of relevant articles (category A; relevant and reliable or reliable with restrictions after detailed assessment) – *initial literature search*

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
49	CA 5.3	Gao H. et al.	2019	Activation of the N-methyl-d-aspartate receptor is involved in glyphosate-induced renal proximal tubule cell apoptosis.	Journal of applied toxicology (2019), Vol. 39, pp. 1096	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.3 for an assessment.
78	CA 5.3	Kumar S. et al.	2014	Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation.	Toxicology (2014), Vol. 325, pp. 42	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.3 for an assessment.
100	CA 5.3	Mesnage R. et al.	2018	Comparison of transcriptome responses to glyphosate, isoxaflutole, quizalofop-p-ethyl and mesotrione in the HepaRG cell line.	Toxicology reports (2018), Vol. 5, pp. 819	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.3 for an assessment.
104	CA 5.3	Milic M. et al.	2018	Oxidative stress, cholinesterase activity, and DNA damage in the liver, whole blood, and plasma of Wistar rats following a 28-day exposure to glyphosate.	Arhiv za higijenu rada i toksikologiju (2018), Vol. 69, No. 2, pp. 154	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
157	CA 5.3	Tang J. et al.	2017	Ion Imbalance Is Involved in the Mechanisms of Liver Oxidative Damage in Rats Exposed to Glyphosate.	Frontiers in physiology (2017), Vol. 8, pp. 1083	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.3 for an assessment.
1	CA 5.4	Adler-Flindt S. et al.	2019	Comparative cytotoxicity of plant protection products and their active ingredients.	Toxicology In Vitro, (2019) Vol. 54, pp. 354	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
31	CA 5.4	da Silva Natara D. G. et al.	2019	Interference of goethite in the effects of glyphosate and Roundup® on ZFL cell line.	Toxicology in vitro (2020), Vol. 65, pp. 104755	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	**The applicant did not provide an assessment of this study. A data gap is identified for the applicant to provide an evaluation of the study including a relevance and reliability assessment of the study.**
37	CA 5.4	de Almeida, L. K. S. et al.	2018	Moderate levels of glyphosate and its formulations vary in their cytotoxicity and genotoxicity in a whole blood model and in human cell lines with different estrogen receptor status.	3 Biotech (2018), Vol. 8, No. 10, pp. 438	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
64	CA 5.4	Ilyushina N. A. et al.	2018	Comparative investigation of genotoxic activity of glyphosate technical products in the micronucleus test in vivo.	Toksikologicheskii Vestnik (2018), No. 4, pp. 24	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
65	CA 5.4	Ilyushina N. A. et al.	2019	Maximum tolerated doses and erythropoiesis effects in the mouse bone marrow by 79 pesticides' technical materials assessed with the micronucleus assay.	Toxicology Reports (2019), Vol. 6, pp. 105	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
73	CA 5.4	Kasuba V. et al.	2017	Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line.	Environmental science and pollution research international (2017), Vol. 24, No. 23, pp. 19267	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
76	CA 5.4	Koller V. J. et al.	2012	Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells.	Archives of toxicology (2012), Vol. 86, No. 5, pp. 805	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
79	CA 5.4	Kwiatkowska M. et al.	2017	DNA damage and methylation induced by glyphosate in peripheral blood mononuclear cells (in vitro study)	Food and chemical toxicology (2017), Vol. 105, pp. 93	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
91	CA 5.4	Manas F. et al.	2013	Oxidative stress and comet assay in tissues of mice administered glyphosate and ampa in drinking water for 14 days.	Journal of Basic and Applied Genetics (2013), Vol. 24, No. 2, pp. 67	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
110	CA 5.4	Nagy K. et al.	2019	Comparative cyto- and genotoxicity assessment of glyphosate and glyphosate-based herbicides in human peripheral white blood cells.	Environmental research (2019), Vol. 179, No. Pt B, pp. 108851	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
131	CA 5.4	Roustan A. et al.	2014	Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation.	Chemosphere (2014), Vol. 108, pp. 93	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
138	CA 5.4	Santovito A. et al.	2018	In vitro evaluation of genomic damage induced by glyphosate on human lymphocytes.	Environmental science and pollution research international (2018), Vol. 25, No. 34, pp. 34693	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
152	CA 5.4	Suarez-Larios K. et al.	2017	Screening of Pesticides with the Potential of Inducing DSB and Successive Recombinational Repair.	Journal of Toxicology (2017), Article ID 3574840	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
165	CA 5.4	Townsend M. et al.	2017	Evaluation of various glyphosate concentrations on DNA damage in human Raji cells and its impact on cytotoxicity.	Regulatory toxicology and pharmacology (2017), Vol. 85, pp. 79	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.4 for an assessment.
6	CA 5.5	Andreotti G. et al.	2018	Glyphosate Use and Cancer Incidence in the Agricultural Health Study	Journal of the national cancer institute (2018) Vol. 110, No. 5, pp. 509	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.
14	CA 5.5	Bisemi M. et al.	2019	Quizalofop-p-Ethyl Induces Adipogenesis in 3T3-L1 Adipocytes.	Toxicological sciences (2019), Vol. 1, No. 170, pp. 452	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.
30	CA 5.5	Crump K.	2020	The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate.	Risk analysis (2020), Vol. 40, pp. 696	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.
44	CA 5.5	Duforestel M. et al.	2019	Glyphosate Primes Mammary Cells for Tumorigenesis by Reprogramming the Epigenome in a TET3-Dependent Manner.	Frontiers in genetics (2019), Vol. 10, pp. 885	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
116	CA 5.5	Pahwa M. et al.	2019	Glyphosate use and associations with non-Hodgkin lymphoma major histological sub-types: findings from the North American Pooled Project.	Scandinavian journal of work, environment & health (2019), Vol. 1; No. 45, pp. 600	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.
123	CA 5.5	Presutti R. et al.	2016	Pesticide exposures and the risk of multiple myeloma in men: An analysis of the North American Pooled Project.	International Journal of Cancer (2016), Vol. 139, No. 8, pp. 1703	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.
149	CA 5.5	Sorahan T.	2015	Multiple myeloma and glyphosate use: a re-analysis of US Agricultural Health Study (AHS) data.	International journal of environmental research and public health (2015), Vol. 12, No. 2, pp. 1548	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.
173	CA 5.5	Wang L. et al.	2019	Glyphosate induces benign monoclonal gammopathy and promotes multiple myeloma progression in mice.	Journal of hematology & oncology, (2019), Vol. 12, No. 1, pp. 70	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.
175	CA 5.5	Wozniak E. et al.	2019	Glyphosate affects methylation in the promoter regions of selected tumor suppressors as well as expression of major cell cycle and apoptosis drivers in PBMCs (in vitro study).	Toxicology in vitro (2019), Vol. 63, pp. 104736	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.
33	CA 5.6	Dai P. et al.	2016	Effect of glyphosate on reproductive organs in male rat.	Acta histochemica (2016), Vol. 118, No. 5, pp. 51	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an assessment.
46	CA 5.6	Forgacs A. L. et al.	2012	BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants.	Toxicological sciences (2012), Vol. 127, No. 2, pp. 391	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an assessment.
56	CA 5.6	Gorga A. et al.	2020	In vitro effects of glyphosate and Roundup on Sertoli cell physiology.	Toxicology in vitro (2020), Vol. 62, pp. 104682	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an assessment.
69	CA 5.6	Johansson H. et al.	2018	Exposure to a glyphosate-based herbicide formulation, but not glyphosate alone, has only minor effects on adult rat testis.	Reproductive toxicology (2018), Vol. 82, pp. 25	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an assessment.
93	CA 5.6	Manservisi F. et al.	2019	The Ramazzini Institute 13-week pilot study glyphosate-based herbicides administered at human-equivalent dose to Sprague Dawley rats: effects on development and endocrine system.	Environmental health (2019), Vol. 18, No. 1, pp. 15	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an assessment.
117	CA 5.6	Panzacchi S. et al.	2018	The Ramazzini Institute 13-week study on glyphosate-based herbicides at humanequivalent dose in Sprague Dawley rats: study design and first in-life endpoints	Environmental Health (2018), Vol. 17, pp. 52/1	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
				evaluation			assessment.
119	CA 5.6	Perego M. C. et al.	2017	Evidence for direct effects of glyphosate on ovarian function: glyphosate influences steroidogenesis and proliferation of bovine granulosa but not theca cells in vitro.	Journal of applied toxicology (2017), Vol. 37, No. 6, pp. 692	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an assessment.
121	CA 5.6	Pham Thu H. et al.	2019	Perinatal Exposure to Glyphosate and a Glyphosate-Based Herbicide Affect Spermatogenesis in Mice.	Toxicological sciences (2019), Vol. 169, No. 1, pp. 260	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an assessment.
128	CA 5.6	Ren Xin et al.	2019	Effects of chronic glyphosate exposure to pregnant mice on hepatic lipid metabolism in offspring.	Environmental pollution (2019), Vol. 254, No. Pt A, pp. 112906	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an assessment.
177	CA 5.6	Zhang J. et al.	2019	The toxic effects and possible mechanisms of glyphosate on mouse oocytes.	Chemosphere (2019), Vol. 237, pp. 124435	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.6.3.1 for an assessment.
22	CA 5.7	Chorfa A. et al.	2013	Specific pesticide-dependent increases in α-synuclein levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines.	Toxicological sciences (2013), Vol. 133, No. 2, pp. 289	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.7 for an assessment.
95	CA 5.7	Martinez A. et al.	2019	Effects of glyphosate and aminomethylphosphonic acid on an isogeneic model of the human blood-brain barrier.	Toxicology letters (2019), Vol. 304, pp. 39	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.7 for an assessment.
96	CA 5.7	Martinez M. A. et al.	2018	Neurotransmitter changes in rat brain regions following glyphosate exposure.	Environmental research (2018), Vol. 161, pp. 212	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.7 for an assessment.
101	CA 5.8	Mesnage R. et al.	2018	Ignoring Adjuvant Toxicity Falsifies the Safety Profile of Commercial Pesticides.	Frontiers in Public Health (2018), Vol. 5, pp. 361	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	in the dossier. Please refer to Volume 3 CA B.6.3 for an assessment.
169	CA 5.8	Vanlaeys A. et al.	2018	Formulants of glyphosate-based herbicides have more deleterious impact than glyphosate on TM4 Sertoli cells.	Toxicology in vitro (2018), Vol. 52, pp. 14.	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.8 for an assessment.
60	CA 5.8.1	Hao Y. et al.	2019	Roundup-Induced AMPK/mTOR-Mediated Autophagy in Human A549 Cells.	Journal of agricultural and food chemistry (2019), Vol. 67, No. 41, pp. 11364	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment.
80	CA 5.8.1	Kwiatkowska M. et al.	2020	Evaluation of apoptotic potential of glyphosate metabolites and impurities in human peripheral blood mononuclear cells (in vitro study).	Food and chemical toxicology (2020) Vol. 135, pp. 110888	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.8 for an assessment.
47	CA 5.8.2	Forsythe S. D. et al.	2018	Environmental Toxin Screening Using Human-Derived 3D Bioengineered Liver and Cardiac Organoids.	Frontiers in public health (2018), Vol. 6, pp. 103	5.4.1 case a) relevant and provides data for the risk assessment:	in the dossier. Please refer to Volume 3 CA B.6.8 for an

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
						Summary is provided in MCA 5	assessment.
52	CA 5.8.3	Gigante P. et al.	2018	Glyphosate affects swine ovarian and adipose stromal cell functions.	Animal reproduction science (2018), Vol. 195, pp. 185	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	in the dossier. Please refer to Volume 3 CA B.6.8 for an assessment.
102	CA 5.8.3	Mesnage R. et al.	2017	Evaluation of estrogen receptor alpha activation by glyphosate-based herbicide constituents.	Food and chemical toxicology (2017) Vol. 108, No. Pt A, pp. 30	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.8 for an assessment.
162	CA 5.8.3	Thongprakaisang S. et al.	2013	Glyphosate induces human breast cancer cells growth via estrogen receptors.	Food and chemical toxicology (2013), Vol. 59, pp. 129	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.8 for an assessment.
23	CA 5.9	Connolly A. et al.	2018	Characterising glyphosate exposures among amenity horticulturists using multiple spot urine samples.	International journal of hygiene and environmental health (2018), Vol. 221, No. 7, pp. 1012	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
24	CA 5.9	Connolly A. et al.	2019	Exploring the half-life of glyphosate in human urine samples.	International journal of hygiene and environmental health (2019), Vol. 222, No. 2, pp. 205	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
25	CA 5.9	Connolly A. et al.	2017	Exposure assessment using human biomonitoring for glyphosate and fluroxypyr users in amenity horticulture.	International journal of hygiene and environmental health (2017), Vol. 220, No. 6, pp. 1064	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
26	CA 5.9	Connolly A. et al.	2018	Glyphosate in Irish adults - A pilot study in 2017.	Environmental research (2018), Vol. 165, pp. 235	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
27	CA 5.9	Connolly A. et al.	2019	Evaluating Glyphosate Exposure Routes and Their Contribution to Total Body Burden: A Study Among Amenity Horticulturalists.	Annals of work exposures and health (2019), Vol. 63, No. 2, pp. 133	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
28	CA 5.9	Conrad A. et al.	2017	Glyphosate in German adults - Time trend (2001 to 2015) of human exposure to a widely used herbicide	International journal of hygiene and environmental health (2017), Vol. 220, No. 1, pp. 8	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
77	CA 5.9	Kongtip P. et al.	2017	Glyphosate and Paraquat in Maternal and Fetal Serums in Thai Women.	Journal of agromedicine (2017), Vol. 22, No. 3, pp. 282	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
98	CA 5.9	McGuire M. K.	2016	Glyphosate and aminomethylphosphonic acid are not	The American journal of	5.4.1 case a) relevant and provides	Publication included in the

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
		et al.		detectable in human milk.	clinical nutrition (2016), Vol. 103, No. 5, pp. 1285	data for the risk assessment: Summary is provided in MCA 5	dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
146	CA 5.9	Sierra-Diaz E. et al.	2019	Urinary pesticide levels in children and adolescents residing in two agricultural communities in Mexico	International Journal of Environmental Research and Public Health (2019), Vol. 16, No. 4, pp. 562	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
150	CA 5.9	Steinborn A. et al.	2016	Determination of Glyphosate Levels in Breast Milk Samples from Germany by LC-MS/MS and GC-MS/MS.	Journal of agricultural and food chemistry (2016), Vol. 64, No. 6, pp. 1414	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.
166	CA 5.9	Trasande L. et al.	2020	Glyphosate exposures and kidney injury biomarkers in infants and young children.	Environmental pollution (2020), Vol. 256, pp. 113334	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	Publication included in the dossier. Please refer to Volume 3 CA B.6.9 for an assessment.

Table B.6.10-1b: Overview of relevant articles (catergory A; relevant and reliable or reliable with restrictions after detailed assessment) – *top up literature search 2020*

Submission Number	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification AGG	RMS comments
6	CA 5.2.6	Lindberg T. *et al.*	2020	An integrated transcriptomic- and proteomic-based approach to evaluate the human skin sensitization potential of glyphosate and its commercial agrochemical formulations.	Journal of proteomics, (2020) Vol. 217, Art. No. 103647	5.4.1 case a) relevant and provides data for the risk assessment: A summary for this article is provided.	Publication included in the dossier. Refer to B.6.2.6
1	CA 5.5	Crump K. *et al.*	2020	Accounting for Multiple Comparisons in Statistical Analysis of the Extensive Bioassay Data on Glyphosate.	Toxicological sciences : an official journal of the Society of Toxicology, (2020) Vol. 175, No. 2, pp. 156-167	5.4.1 case a) relevant and provides data for the risk assessment: A summary is presented in the AIR5 dossier under MCA 5.5./026.	Publication included in the dossier. Refer to B.6.5.18.1
8	CA 5.5	Portier C. J. *et al.*	2020	A comprehensive analysis of the animal carcinogenicity data for glyphosate from chronic exposure rodent carcinogenicity studies.	Environmental health : a global access science source, (2020) Vol. 19, No. 1, pp. 18	5.4.1 case a) relevant and provides data for the risk assessment: A summary is presented in the AIR5 dossier under MCA 5.5./027.	Publication included in the dossier. Refer to B.6.5.18.2
3	CA 5.6	Ganesan S. *et al.*	2020	Absence of glyphosate-induced effects on ovarian folliculogenesis and steroidogenesis.	Reproductive toxicology, (2020) Vol. 96, pp 156-164	5.4.1 case a) relevant and provides data for the risk assessment: A summary for this article is provided.	Publication included in the dossier. Refer to B6.8.3
12	CA 5.8.2	Yahfoufi Z. A. *et al.*	2020	Glyphosate Induces Metaphase II Oocyte Deterioration and Embryo Damage by Zinc Depletion and Overproduction of Reactive Oxygen Species.	Toxicology, (2020) Vol. 439, Art. No. 152466	5.4.1 case a) relevant and provides data for the risk assessment: A summary for this article is provided.	Publication included in the dossier. Refer to B.6.6.3.2.
4	CA 5.8.3	Gastiazoro M. P. et al.	2020	Glyphosate induces epithelial mesenchymal transition-related changes in human endometrial Ishikawa cells via estrogen receptor pathway.	Molecular and cellular endocrinology, (2020) Vol. 510, Art. No. 110841	5.4.1 case a) relevant and provides data for the risk assessment: A summary for this article is provided.	Publication included in the dossier. Refer to B6.8.3
11	CA 5.8.3	Xia Y. et al.	2020	The endoplasmic reticulum stress and related signal pathway mediated the glyphosate-induced testosterone synthesis inhibition in TM3 cells.	Environmental pollution, (2020) Vol. 260, Art. No. 113949	5.4.1 case a) relevant and provides data for the risk assessment: A summary for this article is provided.	Publication included in the dossier. Refer to B6.8.3

Publications excluded from the risk assessment after detailed assessment of full-text documents

The tables below correspond to the publications excluded, not proposed to be included in the assessment, belonging to category B or C or considered not relevant based on full text evaluation.

Category B

Table B.6.10-2a: Overview of relevant but supplementary (category B) articles after detailed assessment – *initial literature search*

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
357	CA 5.1	Hopa E. et al.	2011	The inhibitory effects of some pesticides on human erythrocyte glucose-6-phosphate dehydrogenase activity (in vitro).	Fresenius Environmental Bulletin (2011), Vol. 20, No. 5a, pp. 1314	5.4.1 case b) Relevant but supplementary information: glyphosate and 2,4-D had been used as test material from a "local pesticide shop". No further identification of the test material had been provided, moreover the study design is not well described.	The RMS agrees with the applicant's justification.
421	CA 5.2.1	Lee GaWon et al.	2018	Glyphosate surfactant herbicide toxicosis in a dog with hindlimb paresis and urinary incontinence	Journal of Veterinary Clinics (2018), Vol. 35, No. 4, pp. 144	5.4.1 case b) Relevant but supplementary information: Acute Pet Exposure which should not impact the re-registration.	The RMS agrees with the applicant's justification.
368	CA 5.3	Jasper R. et al.	2012	Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup(®).	Interdisciplinary toxicology (2012), Vol. 5, No. 3, pp. 133	5.4.1 case b) Relevant but supplementary information: Gavaged formulated product, effects not attributable to glyphosate.	The RMS agrees with the applicant's justification.
409	CA 5.3	Larsen K. et al.	2014	Effects of Sublethal Exposure to a Glyphosate-Based Herbicide Formulation on Metabolic Activities of Different Xenobiotic-Metabolizing Enzymes in Rats.	International journal of toxicology (2014), Vol. 33, No. 4, pp. 307	5.4.1 case b) Relevant but supplementary information: Formulation tested in vivo via drinking water (Roundup FULL II, 662 g/L potassium salt). Non-representative formulation for EU.	The RMS agrees with the applicant's justification.
432	CA 5.3	Lieshchova M. A. et al.	2018	Combined effect of glyphosphate, saccharin and sodium benzoate on rats.	Regulatory Mechanisms in Biosystems (2018), Vol. 9, No. 4, pp. 591	5.4.1 case b) Relevant but supplementary information: Substantially lower water consumption in glyphosate only group confounds data and makes endpoint comparisons meaningless.	The RMS agrees with the applicant's justification.
535	CA 5.3	Rebai O. et al.	2017	Morus alba leaf extract mediates neuroprotection against glyphosate-induced toxicity and biochemical alterations in the brain.	Environmental science and pollution research international (2017), Vol. 24, No. 10, pp. 9605	5.4.1 case b) Relevant but supplementary information: Formulation administered via i.p. injection (described as a commercial formulation registered in the Tunisian Ministry of Agriculture).	The RMS agrees with the applicant's justification.
606	CA 5.3	Tizhe E. V. et al.	2014	Influence of zinc supplementation on histopathological changes in the stomach, liver, kidney, brain, pancreas and spleen during subchronic exposure of Wistar rats to glyphosate.	Comparative clinical pathology (2014), Vol. 23, No. 5, pp. 1535	5.4.1 case b) Relevant but supplementary information: Formulation tested (Bushfire, Monsanto Europe, 360 g/L glyphosate; 441 g/L potassium salt). Non-representative formulation for EU.	The RMS agrees with the applicant's justification.
607	CA 5.3	Tizhe E. V. et al.	2013	Haematogical changes induced by	Sokoto Journal of Veterinary	5.4.1 case b) Relevant but supplementary	The RMS agrees with the

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				subchronic glyphosate exposure: ameliorative effect of zinc in Wistar rats.	Sciences (2013), Vol. 11, No. 2, pp. 28	information: Formulation tested in vivo (Bushfire, 441 g/L potassium salt, 360 g/L a.e.). Non-representative formulation for EU.	applicant's justification.
196	CA 5.4	Alvarez-Moya C. et al.	2014	Comparison of the in vivo and in vitro genotoxicity of glyphosate isopropylamine salt in three different organisms.	Genetics and molecular biology (2014),Vol. 37, No. 1, pp. 105	5.4.1 case b) Relevant but supplementary information: Mechanistic study without clear relevance for the risk assessment.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.4 for an assessment of the study.
238	CA 5.4	Brusick D. et al.	2016	Genotoxicity Expert Panel review: weight of evidence evaluation of the genotoxicity of glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid.	Critical reviews in toxicology (2016), Vol. 46, No. sup1, pp. 56	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
250	CA 5.4	Carbajal-Lopez Y. et al.	2016	Biomonitoring of agricultural workers exposed to pesticide mixtures in Guerrero state, Mexico, with comet assay and micronucleus test	Environmental Science and Pollution Research (2016), Vol. 23, No. 3, pp. 2513	5.4.1 case b) Relevant but supplementary information: No glyphosate specific conclusions, confounded due to multiple pesticide uses.	The RMS agrees with the applicant's justification.
286	CA 5.4	de Castilhos Ghisi N. et al.	2016	Does exposure to glyphosate lead to an increase in the micronuclei frequency? A systematic and meta-analytic review.	Chemosphere (2016), Vol. 145, pp. 42	5.4.1 case b) Relevant but supplementary information: No new data presented, only compilation of pooled glyphosate and formulated product meta-analyses.	The RMS agrees with the applicant's justification.
388	CA 5.4	Kier L. D.	2015	Review of genotoxicity biomonitoring studies of glyphosate-based formulations.	Critical reviews in toxicology (2015), Vol. 45, No. 3, pp. 209	5.4.1 case b) Relevant but supplementary information: review, secondary source	The RMS agrees with the applicant's justification.
389	CA 5.4	Kier L. D. et al.	2013	Review of genotoxicity studies of glyphosate and glyphosate-based formulations.	Critical reviews in toxicology (2013), Vol. 43, No. 4, pp. 283	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
437	CA 5.4	Lopez Gonzalez E. C. et al.	2017	Micronuclei and other nuclear abnormalities on Caiman latirostris (Broad-snouted caiman) hatchlings after embryonic exposure to different pesticide formulations.	Ecotoxicology and environmental safety (2017), Vol. 136, pp. 84	5.4.1 case b) Relevant but supplementary information: This study looks at the impact of pesticide formulations on the nuclear developments of Caimen embryos via topical application to their eggs shells after laying. The endpoints achieved cannot be related to EU risk assessment.	The RMS agrees with the applicant's justification.
543	CA 5.4	Rodrigues H. G. et al.	2011	Effects of roundup pesticide on the stability of human erythrocyte membranes and micronuclei frequency in bone marrow cells of Swiss mice	Open Biology Journal (2011), Vol. 4, pp. 54	5.4.1 case b) Relevant but supplementary information: Substance identification is missing, the study is lacking statistically and moreover, a mixed study design has been presented where the micronuclei frequency had been investigated in mice after i.p. injection.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.4 for an assessment of the study.
624	CA 5.4	Vera-Candioti J. et al.	2013	Single-cell gel electrophoresis assay in the ten spotted live-bearer fish,	Ecotoxicology and environmental safety (2013),	5.4.1 case b) Relevant but supplementary information: GBHs tested on fish	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				Cnesterodon decemmaculatus (Jenyns, 1842), as bioassay for agrochemical-induced genotoxicity.	Vol. 98, pp. 368		
183	CA 5.5	Acquavella J. et al.	2018	Corrigendum to: Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma.	Critical Reviews in Toxicology (2018), Vol. 48, No. 10, pp. 898	5.4.1 case b) Relevant but supplementary information: Corrigendum to Acquavella et al._2016, Critical Reviews in Toxicology (2016), Vol. 46, sup1, pp. 28-43.	The RMS agrees with the applicant's justification.
200	CA 5.5	Anon.	2018	Expression of Concern (26 September 2018): An Independent Review of the Carcinogenic Potential of Glyphosate.	Critical Reviews in Toxicology (2018), Vol. 48, No. 10, pp. 981	5.4.1 case b) Relevant but supplementary information: Expression of concern regarding articles Williams et al_2016, Crit Rev Toxicol (2016), 46(S1):3-20 and Solomon et al._2016, Crit Rev Toxicol (2016), 46(S1):21-27 and Acquavella et al_2016, Crit Rev Toxicol (2016), 46(S1):28-43 and Willliams et al._2016, Crit Rev Toxicol (2016), 46(S1):44-55. and Brusick et al_2016, Crit Rev Toxicol (2016), 46(S1):56-74.	The RMS agrees with the applicant's justification.
202	CA 5.5	Arjo G. et al.	2013	Plurality of opinion, scientific discourse and pseudoscience: an in depth analysis of the Seralini et al. study claiming that Roundup® Ready corn or the herbicide Roundup® cause cancer in rats.	Transgenic research (2013), Vol. 22, No. 2, pp. 255	5.4.1 case b) Relevant but supplementary information: Discussion providing context to a controversial retracted publication.	The RMS agrees with the applicant's justification.
220	CA 5.5	Bashir S. et al.	2012	Final review of the Seralini et al. (2012a) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology	EFSA Journal (2012), Vol. 10, No. 11, pp. 2986	5.4.1 case b) Relevant but supplementary information: EFSA review of Seralini chronic rat study.	The RMS agrees with the applicant's justification.
221	CA 5.5	Bashir S. et al.	2012	Review of the Seralini et al. (2012) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology	EFSA Journal (2012), Vol. 10, No. 10, pp. 2910	5.4.1 case b) Relevant but supplementary information: EFSA review of Seralini chronic rat study.	The RMS agrees with the applicant's justification.
226	CA 5.5	Berry C.	2018	The complexities of regulatory toxicology	Outlooks on Pest Management (2018), Vol. 29, No. 6, pp. 270	5.4.1 case b) Relevant but supplementary information: No new data presented.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
227	CA 5.5	Berry C.	2013	Comments on "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize".	Food and Chemical Toxicology (2013), Vol. 53, pp. 430	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comment on Seralini et al._2012_Food Chemical Toxicol. (2012), retracted	The RMS agrees with the applicant's justification.
239	CA 5.5	Brusick D. et al.	2018	Corrigendum to: Genotoxicity Expert Panel review: weight of evidence evaluation of the genotoxicity of glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid.	Critical Reviews in Toxicology (2018), Vol. 46, No. 10, pp 902	5.4.1 case b) Relevant but supplementary information: Corrigendum to Brusick et al._2016, Critical Reviews in Toxicology (2016), Vol. 46, sup1, pp. 56-74	The RMS agrees with the applicant's justification.
240	CA 5.5	Burstyn I. et al.	2017	Visualizing the heterogeneity of effects in the analysis of associations of multiple myeloma with glyphosate use. comments on sorahan, t. multiple myeloma and glyphosate use: A re-analysis of us agricultural health study (AHS) data.	International Journal of Environmental Research and Public Health (2017), Vol. 14, No. 1, pp. 1	5.4.1 case b) Relevant but supplementary information: Re-analysis of old data, no statistically significant glyphosate findings. A re-analysis of US agricultural health study (AHS) data. Int. J. Environ. Res. Public Health (2015), Vol. 12, pp. 1548	The RMS agrees with the applicant's justification.
241	CA 5.5	Bus J. S.	2017	IARC use of oxidative stress as key mode of action characteristic for facilitating cancer classification: Glyphosate case example illustrating a lack of robustness in interpretative implementation.	Regulatory toxicology and pharmacology (2017), Vol. 86, pp. 157	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
310	CA 5.5	Dung Le Tien et al.	2013	Comments on "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize".	Food and Chemical Toxicology (2013), Vol. 53, pp. 428	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comment on Seralini et al._2012_Food Chemical Toxicol (2012), retracted	The RMS agrees with the applicant's justification.
338	CA 5.5	Greim H. et al.	2015	Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies.	Critical reviews in toxicology (2015), Vol. 45, No. 3, pp. 185	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
341	CA 5.5	Grunewald W. et al.	2013	Comment on "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize".	Food and Chemical Toxicology (2013), Vol. 53, pp. 447	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comment on Seralini et al._2012_Food Chemical Toxicol. (2012), retracted	The RMS agrees with the applicant's justification.
349	CA 5.5	Hammond B. et al.	2013	A Comment on "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize".	Food and Chemical Toxicology (2013), Vol. 53, pp. 444	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comment on Seralini et al._2012_Food Chemical Toxicol (2012), retracted	The RMS agrees with the applicant's justification.
353	CA 5.5	Heinemann J. A.	2013	Food and chemical toxicology.	Food and Chemical Toxicology (2013), Vol. 53,	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comment on	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
					pp. 442	Seralini et al._2012_Food Chemical Toxicol (2012), retracted	
378	CA 5.5	Kachuri L. et al.	2013	Multiple pesticide exposures and the risk of multiple myeloma	International Journal of Cancer (2013), Vol. 133, No. 8, pp. 1846	5.4.1 case b) Relevant but supplementary information: Exposure to multiple pesticides and a case control study which is subject to recall bias.	The RMS agrees with the applicant's justification.
415	CA 5.5	Le Tien D. et al.	2013	Comments on "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize"	Food and Chemical Toxicology (2013), Vol. 53, pp. 443	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comment on Seralini et al._2012_Food Chemical Toxicol (2012), retracted	The RMS agrees with the applicant's justification.
449	CA 5.5	McClellan R. O.	2016	Evaluating the potential carcinogenic hazard of glyphosate.	Critical reviews in toxicology (2016), Vol. 46, No. sup1, pp. 1	5.4.1 case b) Relevant but supplementary information: Forward by Editor in Chief to a special edition on glyphosate in Critical Reviews in Toxicology.	The RMS agrees with the applicant's justification.
451	CA 5.5	Mesnage R. et al.	2017	Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide.	Scientific reports (2017), Vol. 7, pp. 39328	5.4.1 case b) Relevant but supplementary information: Formulation tested (Roundup, composition not described). Livers obtained from research of republished retreated Seralini rat study.	The RMS agrees with the applicant's justification.
480	CA 5.5	Nedopitanska N. M.	2011	Problem of the carcinogenic danger of glyphosate; new data	Sovremennye Problemy Toksikologii (2011) No. 1-2, pp. 5	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
486	CA 5.5	Ollivier L.	2013	A Comment on "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize".	Food and Chemical Toxicology (2013), Vol. 53, pp. 458	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comment on Seralini et al._2012_Food Chemical Toxicol (2012), retracted	The RMS agrees with the applicant's justification.
521	CA 5.5	Portier C. J. et al.	2017	Re: Tarazona et al. (2017): Glyphosate toxicity and carcinogenicity: a review of the scientific basis of the European Union assessment and its differences with IARC.	Archives of toxicology (2017), Vol. 91, No. 9, pp. 3195	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, ref to Tarazona et al._2017, Archives of toxicology (2017), Vol. 91, No. 8, pp. 2723-2743.	The RMS agrees with the applicant's justification.
540	CA 5.5	Resnik D. B.	2015	Retracting Inconclusive Research: Lessons from the Seralini GM Maize Feeding Study	Journal of agricultural & environmental ethics (2015), Vol. 28, No. 4, pp. 621	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comment on Seralini et al._2012_Food Chemical Toxicol (2012), retracted	The RMS agrees with the applicant's justification.
561	CA 5.5	Schinasi L. et al.	2014	Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis.	International journal of environmental research and public health (2014), Vol. 11, No. 4, pp. 4449	5.4.1 case b) Relevant but supplementary information: This paper concerns a meta-analysis where the results were taken from available studies at face value. The authors had no way to correct for recall bias, confounding, etc. As the meta-RRs of the studies included are in error the meta-analyses are also in error. The study is considered unreliable.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.5 for an assessment of the study.
564	CA 5.5	Seralini G-E. et	2013	Answers to critics: Why there is a	Food and Chemical	5.4.1 case b) Relevant but supplementary	The RMS agrees with the

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
		al.		long term toxicity due to a Roundup-tolerant genetically modified maize and to a Roundup herbicide	Toxicology (2013), Vol. 53, pp. 476	information: Author responding to multiple Letters to the Editor.	applicant's justification.
579	CA 5.5	Solomon K. R.	2017	What is the problem with glyphosate?	Outlooks on Pest Management (2017), Vol. 28, No. 4, pp. 173	5.4.1 case b) Relevant but supplementary information: Review of IARC deficiencies.	The RMS agrees with the applicant's justification.
581	CA 5.5	Solomon K.R.	2018	Corrigendum to: Glyphosate in the general population and in applicators: a critical review of studies on exposures.	Critical Reviews in Toxicology (2018), Vol 48, No 10, pp. 896	5.4.1 case b) Relevant but supplementary information: Corrigendum to Solomon et al._2016, Critical Reviews in Toxicology (2016), 46, sup1, pp. 21-27.	The RMS agrees with the applicant's justification.
585	CA 5.5	Sorahan T.	2016	Visualising and thinking and interpreting. Response to the Burstyn and de Ros comments on Sorahan "Multiple myeloma and glyphosate use: A re-analysis of us agricultural health study (AHS) data".	International Journal of Environmental Research and Public Health (2016), Vol. 14, No. 1, pp. E6	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Response to Burstyn et al. on Sorahan et al._2015, Int. J. Environ. Res. Public Health (2015), Vol. 12, pp. 1548-1559.	The RMS agrees with the applicant's justification.
592	CA 5.5	Stipicevic S.	2017	Some organophosphate insecticides and herbicides	Arhiv Za Higijenu Rada i Toksikologiju (2017), Vol. 68, No. 2, pp. A10	5.4.1 case b) Relevant but supplementary information: Commentary on IARC evaluation.	The RMS agrees with the applicant's justification.
600	CA 5.5	Tarazona J. V. et al.	2017	Glyphosate toxicity and carcinogenicity: a review of the scientific basis of the European Union assessment and its differences with IARC.	Archives of toxicology (2017), Vol. 91, No. 8, pp. 2723	5.4.1 case b) Relevant but supplementary information: Comparison of EU regulatory review with IARC evaluation.	The RMS agrees with the applicant's justification.
601	CA 5.5	Tarazona J. V. et al.	2017	Response to the reply by C. J. Portier and P. Clausing, concerning our review "Glyphosate toxicity and carcinogenicity: a review of the scientific basis of the European Union assessment and its differences with IARC".	Archives of toxicology (2017), Vol. 91, No. 9, pp. 3199	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, ref to Portier et al_2017_Arch Toxicol (2017), Vol. 91, No. 9, pp. 3195-3197.	The RMS agrees with the applicant's justification.
602	CA 5.5	Tarone R. E.	2018	On the International Agency for Research on Cancer classification of glyphosate as a probable human carcinogen	European journal of cancer prevention (2018), Vol. 27, No. 1, pp. 82	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
611	CA 5.5	Tribe D.	2013	Serious inadequacies regarding the pathology data presented in the paper by Seralini et al. (2012).	Food and Chemical Toxicology (2013), Vol. 53, pp. 452	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comment on Seralini et al._2012_Food Chemical Toxicol (2012), retracted.	The RMS agrees with the applicant's justification.
634	CA 5.5	Williams G. M.	2018	Corrigendum to: Glyphosate rodent carcinogenicity bioassay expert panel review (Critical Reviews in	Critical Reviews in Toxicology (2018), Vol. 48, No. 10, pp. 914	5.4.1 case b) Relevant but supplementary information: Corrigendum to article Williams_2016, Critical reviews in toxicology (2016), Vol. 46, No.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				Toxicology, (2016), 46, sup1, (44-55), 10.1080/10408444.2016.1214679)		sup1, pp. 4	
635	CA 5.5	Williams G. M. et al.	2016	Glyphosate rodent carcinogenicity bioassay expert panel review.	Critical reviews in toxicology (2016), Vol. 46, No. sup1, pp. 44	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
636	CA 5.5	Williams G. M. et al.	2018	Corrigendum: A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment.	Critical Reviews in Toxicology (2018), Vol. 48, No. 10, pp. 907	5.4.1 case b) Relevant but supplementary information: Corrigendum to: A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment (Critical Reviews in Toxicology, (2016), 46, sup1, pp. 3-20.)	The RMS agrees with the applicant's justification.
182	CA 5.6	Abou-Amer W. L. et al.	2010	Teratological effects induced by three pesticides in pregnant rats	Alexandria Journal of Pharmaceutical Sciences (2010), Vol. 24, No. 1, pp. 21	5.4.1 case b) Relevant but supplementary information: Supportive only: Study is done with pesticide formulations with only one dose per pesticide treatment group established. The study contains unsufficient data, therefore supplementary only.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.6.3.2 for an assessment of the study.
224	CA 5.6	Belle R. et al.	2012	Letter to the Editor: Toxicity of Roundup and glyphosate.	Journal of Toxicology and Environmental Health Part B Critical Reviews (2012), Vol. 15, No. 4, pp. 233	5.4.1 case b) Relevant but supplementary information: Response to Letter to the Editor, comments on Williams et al_2012, J. Toxicol. nviron. Health B Crit. Rev (2012), Vol. 15, No. 1, pp. 39-96.	The RMS agrees with the applicant's justification.
246	CA 5.6	Cai W. et al.	2017	Effects of glyphosate exposure on sperm concentration in rodents: A systematic review and meta-analysis.	Environmental toxicology and pharmacology (2017), Vol. 55, pp. 148	5.4.1 case b) Relevant but supplementary information: Re-evaluation of pooled literature data.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.6.3.1 for an assessment of the study.
282	CA 5.6	de Almeida L. L. et al.	2017	Effects of melatonin in rats in the initial third stage of pregnancy exposed to sub-lethal doses of herbicides.	Acta histochemica (2017), Vol. 119, No. 3, pp. 220	5.4.1 case b) Relevant but supplementary information: Formulation tested at high doses of 500 mg/kg bw/day (Roundup), therefore supplementary only.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.6.3.2 for an assessment of the study.
293	CA 5.6	Defarge N. et al.	2012	Letter to the Editor: Developmental and reproductive outcomes of Roundup and Glyphosate in humans and animals.	Journal of Toxicology and Environmental Health Part B Critical Reviews (2012), Vol. 15, No. 7, pp. 433	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, reaction on Williams et al._2012, Toxicol. Environ. Health B Crit. Rev. 15(1):39-96.	The RMS agrees with the applicant's justification.
299	CA 5.6	DeSesso J. M. et al.	2012	Letter to the Editor: Toxicity of Roundup and Glyphosate response.	Journal of Toxicology and Environmental Health Part B Critical Reviews (2012), Vol. 15, No. 4, pp. 236	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, response on Belle_2012, Journal of Toxicology and Environmental Health Part B Critical Reviews, (2012) Vol. 15, No. 4, pp. 233-235.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
300	CA 5.6	DeSesso J. M. et al.	2012	Comment on "Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression".	Archives of Toxicology (2012), Vol. 86, No. 11, pp. 1791	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comments on Romano et al._2012, Arch Toxicol (2012), Vol. 86, No. 4, pp. 663-73.	The RMS agrees with the applicant's justification.
301	CA 5.6	DeSesso J. M. et al.	2012	Response to the comments of Defarge and colleagues.	Journal of Toxicology and Environmental Health Part B Critical Reviews (2012), Vol. 15, No. 7, pp. 438	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, reaction on Defarge et al._2012_Journal of Toxicology and Environmental Health Part B Critical Reviews (2012), Vol. 15, No. 7, pp. 433-437.	The RMS agrees with the applicant's justification.
446	CA 5.6	Manfo F. P. T. et al.	2012	Effect of agropesticides use on male reproductive function: A study on farmers in Djutitsa (Cameroon)	Environmental Toxicology (2012), Vol. 27, No. 7, pp. 423	5.4.1 case b) Relevant but supplementary information: No glyphosate specific conclusions, confounded due to multiple pesticide uses.	The RMS agrees with the applicant's justification.
490	CA 5.6	Owagboriaye F. O. et al.	2017	Reproductive toxicity of Roundup herbicide exposure in male albino rat.	Experimental and toxicologic pathology (2017), Vol. 69, No. 7, pp. 461	5.4.1 case b) Relevant but supplementary information: Formulation tested in vivo (Roundup 441 g/L potassium salt, 360 g/L a.e.).	RMS requested a study summary in order to further justify the categorization. Refer to B.6.6.3.1 for an assessment of the study.
551	CA 5.6	Sakpa C. L. et al.	2018	Effects of glyphosate on sperm parameters and pregnancy success rate in Wistar rats.	Annals of Biomedical Sciences (2018), Vol. 17, No. 2, pp. 156	5.4.1 case b) Relevant but supplementary information: The glyphosate used is not sufficiently characterized, only two dose levels were tested and the number of animals used per dose level was too low. This publication is considered unreliable.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.6.3.1 for an assessment of the study.
632	CA 5.6	Williams A. L. et al.	2012	Developmental and reproductive outcomes in humans and animals after glyphosate exposure: a critical analysis.	Journal of toxicology and environmental health. Part B, Critical reviews (2012), Vol. 15, No. 1, pp. 39	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
457	CA 5.6.1	Milesi M. M. et al.	2018	Perinatal exposure to a glyphosate-based herbicide impairs female reproductive outcomes and induces second-generation adverse effects in Wistar rats.	Archives of toxicology (2018), Vol. 92, No. 8, pp. 2629	5.4.1 case b) Relevant but supplementary information: Glyphosate based herbicide (54% glyphosate acid equivalents as the K salt) dosed to pregnant rats.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.6.3.1 for an assessment of the study.
458	CA 5.6.1	Milesi M. M. et al.	2019	Response to comments on: Perinatal exposure to a glyphosate-based herbicide impairs female reproductive outcomes and induces second-generation adverse effects in Wistar rats.	Archives of toxicology (2019), Vol. 93, No. 12, pp. 3635	5.4.1 case b) Relevant but supplementary information: Glyphosate based herbicide (54% glyphosate acid equivalents as the K salt) dosed to pregnant rats.	Refer to B.6.6.3.1 for an assessment of the study (refer to Milesi *et al*., 2018).
518	CA 5.6.1	Plewis I.	2019	Comment on: Perinatal exposure to a glyphosate-based herbicide impairs female reproductive outcomes and induces second-generation adverse effects in Wistar rats.	Archives of toxicology (2019), Vol. 93, No. 1, pp. 207	5.4.1 case b) Relevant but supplementary information: Glyphosate based herbicide (54% glyphosate acid equivalents as the K salt) dosed to pregnant rats.	Refer to B.6.6.3.1 for an assessment of the study (refer to Milesi *et al*., 2018).

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
519	CA 5.6.1	Plewis I.	2020	Comment on response from Milesi et al. to 'Perinatal exposure to a glyphosate-based herbicide impairs female reproductive outcomes and induces second-generation adverse effects in Wistar rats'.	Archives of toxicology (2020), Vol. 94, pp. 351	5.4.1 case b) Relevant but supplementary information: Glyphosate based herbicide (54% glyphosate acid equivalents as the K salt) dosed to pregnant rats.	Refer to B.6.6.3.1 for an assessment of the study (refer to Milesi *et al*., 2018).
623	CA 5.6.1	Velastegui-Espin G. P. et al.	2018	Glyphosate: its use and implications for human health. El glifosato: su uso e implicaciones en la salud humana.	Journal of the Selva Andina Biosphere (2018), Vol. 6, No. 2, pp. 86	5.4.1 case b) Relevant but supplementary information: review, secondary source of information.	The RMS agrees with the applicant's justification.
394	CA 5.6.2	Kimmel G. L. et al.	2013	Evaluation of developmental toxicity studies of glyphosate with attention to cardiovascular development.	Critical reviews in toxicology (2013), Vol. 43, No. 2, pp. 79	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
318	CA 5.7	Feldman V.	2014	Neurodevelopmental toxicity: Still more questions than answers.	The Lancet Neurology (2014), Vol. 13, No. 7, pp. 645	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comments on Grandjean et al_2014, Lancet Neurol. 2014 Jul;13(7):648-9.	The RMS agrees with the applicant's justification.
333	CA 5.7	Goldstein D. A. et al.	2014	Neurodevelopmental toxicity: Still more questions than answers.	The Lancet Neurology (2014), Vol. 13, No. 7, pp. 645	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comments on Grandjean et al._2014, Lancet Neurol (2014), Vol. 13, No. 7, pp. 648-9.	The RMS agrees with the applicant's justification.
337	CA 5.7	Grandjean P. et al.	2014	Neurodevelopmental toxicity: Still more questions than answers - Authors' response.	The Lancet Neurology (2014), Vol. 13, No. 7, pp. 648	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, author responding to multiple Letters to Editors	The RMS agrees with the applicant's justification.
189	CA 5.8	Ait Bali Y. et al.	2017	Behavioral and Immunohistochemical Study of the Effects of Subchronic and Chronic Exposure to Glyphosate in Mice.	Frontiers in behavioral neuroscience (2017), Vol. 11, pp. 146	5.4.1 case b) Relevant but supplementary information: Formulation tested (Roundup, 486 g/L isopropylamine salt, 360 g/L a.e.) in vivo.	The RMS agrees with the applicant's justification.
215	CA 5.8	Baier C. J. et al.	2017	Behavioral impairments following repeated intranasal glyphosate-based herbicide administration in mice.	Neurotoxicology and teratology (2017), Vol. 64, pp. 63	5.4.1 case b) Relevant but supplementary information: Formulation tested via intranasal administration.	The RMS agrees with the applicant's justification.
248	CA 5.8	Caloni F. et al.	2016	Suspected poisoning of domestic animals by pesticides.	The Science of the total environment (2016), Vol. 539, pp. 331	5.4.1 case b) Relevant but supplementary information: Review article on domestic animal poisonings by pesticides.	The RMS agrees with the applicant's justification.
284	CA 5.8	de Avila R. I. et al.	2017	In vitro assessment of skin sensitization, photosensitization and phototoxicity potential of commercial glyphosate-containing formulations.	Toxicology in vitro (2017), Vol. 45, No. 3, pp. 386	5.4.1 case b) Relevant but supplementary information: Non-validated model confirms glyphosate non-sensitized & non-photosensitizer. Formulation data inconsistent in non-validated model.	The RMS agrees with the applicant's justification.
294	CA 5.8	Defarge N. et al.	2016	Co-Formulants in Glyphosate-Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels.	International journal of environmental research and public health (2016), Vol. 13, No. 3, pp. 264	5.4.1 case b) Relevant but supplementary information: In vitro results not significant for glyphosate vs multiple formulations or mixtures.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
317	CA 5.8	Farkas E. et al.	2018	Label-free optical biosensor for real-time monitoring the cytotoxicity of xenobiotics: A proof of principle study on glyphosate.	Journal of hazardous materials (2018), Vol. 351, pp. 80	5.4.1 case b) Relevant but supplementary information: in vitro cytotoxicity assays.	The RMS agrees with the applicant's justification.
339	CA 5.8	Gress S. et al.	2015	Glyphosate-based herbicides potently affect cardiovascular system in mammals: review of the literature.	Cardiovascular toxicology (2015), Vol. 15, No. 2, pp. 117	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
342	CA 5.8	Gui Y-X. et al.	2012	Glyphosate induced cell death through apoptotic and autophagic mechanisms.	Neurotoxicology and teratology (2012), Vol. 34, No. 3, pp. 344	5.4.1 case b) Relevant but supplementary information: Unrealistically high in vitro dosing in the mM range.	The RMS agrees with the applicant's justification.
393	CA 5.8	Kim Y-h.. et al.	2013	Mixtures of glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis.	Toxicology in vitro : an international journal published in association with BIBRA (2013), Vol. 27, No. 1, pp. 191	5.4.1 case b) Relevant but supplementary information: In vitro cytotoxicity endpoints measured for glyphosate & surfactant along and in combination. No significant effects with glyphosate alone.	The RMS agrees with the applicant's justification.
402	CA 5.8	Kurenbach B. et al.	2015	Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium.	mBio (2015), Vol. 6, No. 2, pp. E00009	5.4.1 case b) Relevant but supplementary information: Endpoints at doses tested not relevant to resides levels or to human health.	The RMS agrees with the applicant's justification.
403	CA 5.8	Kwiatkowska M. et al.	2014	The effect of glyphosate, its metabolites and impurities on erythrocyte acetylcholinesterase activity.	Environmental toxicology and pharmacology (2014), Vol. 37, No. 3, pp. 1101	5.4.1 case b) Relevant but supplementary information: In vitro effects only noted at excessively high doses, 250-5000 uM.	The RMS agrees with the applicant's justification.
452	CA 5.8	Mesnage R. et al.	2013	Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity.	Toxicology (2013), Vol. 313, No. 2-3, pp. 122	5.4.1 case b) Relevant but supplementary information: Formulations, surfactants and glyphosate tested in vitro. Effects attributable to surfactant cytotoxicity.	The RMS agrees with the applicant's justification.
453	CA 5.8	Mesnage R. et al.	2017	Facts and Fallacies in the Debate on Glyphosate Toxicity.	Frontiers in public health (2017), Vol. 5, pp. 316	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
454	CA 5.8	Mesnage R. et al.	2014	Major pesticides are more toxic to human cells than their declared active principles.	BioMed research international (2014), Vol. 2014, pp. 179691	5.4.1 case b) Relevant but supplementary information: In vitro cytotoxicity data at high doses not informative for hazard characterization.	The RMS agrees with the applicant's justification.
553	CA 5.8	Saltmiras D. A. et al.	2015	Glyphosate: The Fate and Toxicology of a Herbicidal Amino Acid Derivative.	Amino Acids in Higher Plants (2015), pp. 461	5.4.1 case b) Relevant but supplementary information: Overview of glyphosate toxicology and fate data.	The RMS agrees with the applicant's justification.
584	CA 5.8	Song H-Y. et al.	2012	In vitro cytotoxic effect of glyphosate mixture containing surfactants.	Journal of Korean medical science (2012), Vol. 27, No. 7, pp. 711	5.4.1 case b) Relevant but supplementary information: In vitro mixture effects only, not glyphosate alone.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
194	CA 5.8.2	Alleva R. et al.	2018	Mechanism underlying the effect of long-term exposure to low dose of pesticides on DNA integrity.	Environmental Toxicology (2018), Vol. 33, No. 4, pp. 476	5.4.1 case b) Relevant but supplementary information: Purity and source not reported. No positive control. Only one or two concentrations of glyphosate were tested. Comparisons are to untreated cells rather than negative controls. The reliability of the study is unassignable.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.
198	CA 5.8.2	Andreotti G. et al.	2012	The interaction between pesticide use and genetic variants involved in lipid metabolism on prostate cancer risk	Journal of Cancer Epidemiology (2012), Article ID 358076, pp 1	5.4.1 case b) Relevant but supplementary information: Mechanism of measuring toxicity is not data requirement of (EC) 1107/2009; performed in a non-relevant test model.	The RMS agrees with the applicant's justification.
199	CA 5.8.2	Anifandis G. et al.	2018	The effect of glyphosate on human sperm motility and sperm DNA fragmentation	International Journal of Environmental Research and Public Health (2018), Vol. 15, No. 6, pp. 1117/1	5.4.1 case b) Relevant but supplementary information: The glyphosate used is not characterized, only one test concentration was used, no positive control was considered and the results obtained are not corroborated by in vivo regulatory reproductive toxicology studies with much higher systemic levels of glyphosate. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
290	CA 5.8.2	Dechartres J. et al.	2019	Glyphosate and glyphosate-based herbicide exposure during the peripartum period affects maternal brain plasticity, maternal behaviour and microbiome	Journal of Neuroendocrinology (2019), Vol. 31, pp. e12731	5.4.1 case b) Relevant but supplementary information: The glyphosate used was not sufficiently characterised, only one dose level was tested, the number of animals used per dose level was too low (n = 7) and a unreliable technique for oral dosing was employed (injection of test item in cookies). This publication is considered unreliable.	The RMS agrees with the applicant's justification.
291	CA 5.8.2	Dedeke G. A. et al.	2018	Comparative Assessment on Mechanism Underlying Renal Toxicity of Commercial Formulation of Roundup Herbicide and Glyphosate Alone in Male Albino Rat.	International Journal of Toxicology (2018), Vol. 37, No. 4, pp. 285	5.4.1 case b) Relevant but supplementary information: The glyphosate used was not sufficiently characterized, the number of animals used per dose level was too low, and the conduct of the biochemical tests and the analysis of glyphosate in kidney tissue was poorly described. Moreover, the results from the testing of the oxidative stress parameters seem not reliable. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
328	CA 5.8.2	Gencer N. et al.	2012	In vitro effects of some herbicides and fungicides on human erythrocyte carbonic anhydrase activity	Fresenius Environmental Bulletin (2012), Vol. 21, No. 3, pp. 549	5.4.1 case b) Relevant but supplementary information: Glyphosate tested was not sufficiently characterised, the conditions of the inhibition assay are incompletely reported, no positive control was used and the statistics arenot well reported. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
356	CA 5.8.2	Honskii Y. I. et al.	2011	Effects of heavy metal salts and organophosphoric pesticides on	Medichna Khimiya (2011), Vol. 13, No. 4, pp. 100	5.4.1 case b) Relevant but supplementary information: Mechanistic study without clear	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				protein metabolism in exposed white rats		relevance for the risk assessment / glyphosate.	
410	CA 5.8.2	Larsen K. et al.	2012	Effects of sub-lethal exposure of rats to the herbicide glyphosate in drinking water: glutathione transferase enzyme activities, levels of reduced glutathione and lipid peroxidation in liver, kidneys and small intestine.	Environmental toxicology and pharmacology (2012), Vol. 34, No. 3, pp. 811	5.4.1 case b) Relevant but supplementary information: Only 2 dose levels were used with only 4 animals per sex and per group. Effects were found on GSH in liver at sub-mg/kg bw dose levels which is not concordant with liver effects seen in regulatory toxicology studies performed at much higher dose levels. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
424	CA 5.8.2	Lemma T. et al.	2019	Disruption of giant unilamellar vesicles mimicking cell membranes induced by the pesticides glyphosate and picloram	Biophysical chemistry (2019), Vol. 250, pp. 106176	5.4.1 case b) Relevant but supplementary information: Novel assays and endpoints not applicable/reliable for risk assessment.	The RMS agrees with the applicant's justification.
455	CA 5.8.2	Mesnage R. et al.	2015	Potential toxic effects of glyphosate and its commercial formulations below regulatory limits.	Food and chemical toxicology (2015), Vol. 84, pp. 133	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
489	CA 5.8.2	Owagboriaye F. et al.	2019	Comparative studies on endogenic stress hormones, antioxidant, biochemical and hematological status of metabolic disturbance in albino rat exposed to roundup herbicide and its active ingredient glyphosate.	Environmental science and pollution research international (2019), Vol. 26, No. 14, pp. 14502	5.4.1 case b) Relevant but supplementary information: Purity not reported. Test species are not clearly and completely described. Insufficient information is given on the biochemical methods used. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
534	CA 5.8.2	Razi M. et al.	2012	Histological and histochemical effects of Gly-phosate on testicular tissue and function.	Iranian Journal of Reproductive Medicine (2012), Vol. 10, No. 3, pp. 181	5.4.1 case b) Relevant but supplementary information: No internationally accepted methods were used, only one dose level was considered, there was no characterisation of the test compound and the results are not corroborated by regulatory reproductive toxicity studies using much higher dose levels and longer times of exposure. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
537	CA 5.8.2	Ren X. et al.	2018	Effects of glyphosate on the ovarian function of pregnant mice, the secretion of hormones and the sex ratio of their fetuses.	Environmental pollution (2018), Vol. 243, No. Pt B, pp. 833	5.4.1 case b) Relevant but supplementary information: Glyphosate purity not reported. Only one dose level for glyphosate was tested (0.5% solution added to drinking water (it is unclear what actual dose was administered per day)). The number of animals used per dose level was too low. Insufficient information is given on the biochemical methods used. This publication is considered unreliable.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.
639	CA 5.8.2	Wrobel M. H.	2018	Glyphosate affects the secretion of regulators of uterine contractions in cows while it does not directly impair	Toxicology and applied pharmacology (2018), Vol. 349, pp. 55	5.4.1 case b) Relevant but supplementary information: Glyphosate used is not sufficiently characterized and the analysis of glyphosate,	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				the motoric function of myometrium in vitro.		hormones and prostaglandins is not sufficiently documented. This publication is considered unreliable.	
666	CA 5.8.2	Zhao W. et al.	2011	Effect of glyphosate on oxidative damage of mice	Dulixue Zazhi (2011), Vol. 25, No. 5, pp. 364	5.4.1 case b) Relevant but supplementary information: No new information relevant for the risk assessment.	The RMS agrees with the applicant's justification.
235	CA 5.8.3	Brennan J. C. et al.	2016	Development of a recombinant human ovarian (BG1) cell line containing estrogen receptor α and β for improved detection of estrogenic/antiestrogenic chemicals	Environmental Toxicology and Chemistry (2016), Vol. 35, No. 1, pp. 91	5.4.1 case b) Relevant but supplementary information: Limited data on glyphosate.	The RMS agrees with the applicant's justification.
306	CA 5.8.3	Drasar P. et al.	2018	Glyphosate, an important endocrine disruptor Glyfosat - Dulezity endokrinni disruptor.	Diabetologie Metabolismus Endokrinologie Vyziva (2018), Vol. 21, No. 2, pp. 93	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
346	CA 5.8.3	Haggard D. E. et al.	2018	Erratum to High-Throughput H295R Steroidogenesis Assay: Utility as an Alternative and a Statistical Approach to Characterize Effects on Steroidogenesis.	Toxicological Sciences (2018), Vol. 164, No. 2, pp. 646	5.4.1 case b) Relevant but supplementary information: Erratum to Haggard et al._2018, Toxicological Sciences (2018), Vol. 162, No. 2, pp. 509-534.	The RMS agrees with the applicant's justification.
347	CA 5.8.3	Haggard D. E. et al.	2018	High-throughput H295R steroidogenesis assay: utility as an alternative and a statistical approach to characterize effects on steroidogenesis	Toxicological Sciences (2018), Vol. 162, No. 2, pp. 509	5.4.1 case b) Relevant but supplementary information: ToxCast data for high throughput H295R assay not available on glyphosate, presumably because it is not soluble in DMSO.	The RMS agrees with the applicant's justification.
496	CA 5.8.3	Palma G.	2011	Letter to the editor regarding the article by Paganelli et al.	Chemical research in toxicology (2011), Vol. 24, No. 6, pp. 775	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, reply to Paganelli et al._2010, Chem. Res. Toxicol. (2010), Vol. 23, pp. 1586-1595.	The RMS agrees with the applicant's justification.
498	CA 5.8.3	Pandey A. et al.	2015	Analysis of endocrine disruption effect of Roundup(®) in adrenal gland of male rats.	Toxicology reports (2015), Vol. 2, pp. 1075	5.4.1 case b) Relevant but supplementary information: Formulation tested in vivo (Roundup, 41%, India).	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.3 for an assessment of the study.
515	CA 5.8.3	Pinto C. L. et al.	2018	Identification of candidate reference chemicals for in vitro steroidogenesis assays	Toxicology In Vitro (2018), Vol. 47, pp. 103	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
587	CA 5.8.3	Sritana N. et al.	2018	Glyphosate induces growth of estrogen receptor alpha positive cholangiocarcinoma cells via non-genomic estrogen receptor/ERK1/2 signaling pathway.	Food and chemical toxicology (2018), Vol. 118, pp. 595	5.4.1 case b) Relevant but supplementary information: The results showed that glyphosate has the same potency as Estradiol (E2) when tested at extremely low concentrations.This has not been corroborated by other ED studies. This publication is considered unreliable.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.3 for an assessment of the study.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
665	CA 5.8.3	Zhao H. et al.	2018	Effects of Glyphosate on Testosterone Synthesis in Male Rats.	Asian Journal of Ecotoxicology (2018), Vol. 13, No. 5, pp. 242	5.4.1 case b) Relevant but supplementary information: Reporting of the experimental conditions is not complete.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.3 for an assessment of the study.
217	CA 5.9	Bando H. et al.	2010	Extreme hyperkalemia in a patient with a new glyphosate potassium herbicide poisoning: report of a case.	The Japanese journal of toxicology (2010), Vol. 23, No. 3, pp. 246	5.4.1 case b) Relevant but supplementary information: This case report describes severe hyperkalemia in the setting of suicidal ingestion of potassium salt glyphosate formulations. This is not unexpected.	The RMS agrees with the applicant's justification.
228	CA 5.9	Beswick E. et al.	2011	Fatal poisoning with glyphosate-surfactant herbicide.	Journal of the Intensive Care Society (2011), Vol. 12, No. 1, pp. 37	5.4.1 case b) Relevant but supplementary information: This is a case of a young man who deliberately ingested glyphosate product at home and rapidly developed multi-organ failure, culminating in death. No new observations.	The RMS agrees with the applicant's justification.
264	CA 5.9	Chau A. M. T. et al.	2011	More Data on the Effect of Haemoperfusion for Acute Poisoning Is Required.	Blood Purification (2011), Vol. 31, No. 1-3, pp. 41	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comments on Gil et al_2010, Blood Purif (2010), Vol. 30, No. 2, pp. 84-8.	The RMS agrees with the applicant's justification.
350	CA 5.9	Han S. K. et al.	2010	Use of a lipid emulsion in a patient with refractory hypotension caused by glyphosate-surfactant herbicide.	Clinical toxicology (2010), Vol. 48, No. 6, pp. 566	5.4.1 case b) Relevant but supplementary information: This is a case report of a suicidal ingestion of formulated glyphosate that was treated with lipid emulsion and symptoms improved. As this is a description of medical management of a suicidal overdose, this should not impact re-registration	The RMS agrees with the applicant's justification.
444	CA 5.9	Malhotra R. C. et al.	2010	Glyphosate-surfactant herbicide-induced reversible encephalopathy.	Journal of clinical neuroscience (2010), Vol. 17, No. 11, pp. 1472	5.4.1 case b) Relevant but supplementary information: This paper describes prolonged encephalopathy in a suicidal glyphosate ingestion. There is no mention of the medication that was used for sedation while the patient was intubated in the ICU. Accumulations of lorazepam and other sedatives may result in prolonged coma. In formulated glyphosate overdose with multi-organ failure it is common to sedate patients until their haemodynamics improve. As this document encompasses suicidal overdose, this paper should not impact re-registration.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
469	CA 5.9	Moon J. M. et al.	2010	Predicting acute complicated glyphosate intoxication in the emergency department.	Clinical toxicology (2010), Vol. 48, No. 7, pp. 718	5.4.1 case b) Relevant but supplementary information: The results of this study showed that age > 50 years, X-ray abnormalities, and ALT > 40 U/L were significant predictive factors for complications in patients with glyphosate surfactant herbicide poisoning; patients with these findings might require admission to the intensive care unit.	The RMS agrees with the applicant's justification.
497	CA 5.9	Pan LiPing et al.	2016	Analysis of liver index of workers exposed to glyphosate	Journal of Environmental & Occupational Medicine (2016), Vol. 33, No. 4, pp. 380	5.4.1 case b) Relevant but supplementary information: This article examined the liver function in 345 workers exposed to glyphosate through manufacturing and 345 controls. The sample size is small, and it was claimed that there was a statitically signigicant difference between cholinesterase levels between groups. This is not related to glyphosate as it is not a cholinesterase inhibitor. It was also found that there were markers of liver pathology on ultrasound, which wouldn't be related to glyphosate as this has been extensively evaluated through GLP studies.	The RMS agrees with the applicant's justification.
505	CA 5.9	Park J-S. et al.	2013	Incidence, etiology, and outcomes of rhabdomyolysis in a single terfiary referral center	Journal of Korean Medical Science (2013), Vol. 28, No. 8, pp. 1194	5.4.1 case b) Relevant but supplementary information: This article only mentions glyphosate in the reference section. One reference specifically discusses rhabdomyolysis with intramuscular injection of formulated glyphosate.	The RMS agrees with the applicant's justification.
542	CA 5.9	Roberts D. M. et al.	2010	A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning.	Clinical toxicology (2010), Vol. 48, No. 2, pp. 129	5.4.1 case b) Relevant but supplementary information: This paper is a prospective study of outcomes of suicidal ingestions of glyphosate based herbicides. It shows that the mortality rate from overdose is 3.2%. This paper supports the idea that low-toxicity pesticides have a lower mortality rate than higher toxicity products.	The RMS agrees with the applicant's justification.
560	CA 5.9	Sato C. et al.	2011	Aseptic meningitis in association with glyphosate-surfactant herbicide poisoning.	Clinical toxicology (2011), Vol. 49, No. 2, pp. 118	5.4.1 case b) Relevant but supplementary information: This article evaluates the case of a woman who presented in multi-organ failure 2 days after a formulated glyphosate overdose. Meningitis was suspected and the patient was found to have a high level of glyphosate in CSF. The claim is that glyphosate can cause aseptic meningitis and neurotoxicity. Glyphosate is hydrophilic and cannot cross cell membranes without active transport. It is well known that hypoxia and inflammatory changes can disrupt the tight junctions of the blood brain	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
						barrier which may allow passage of substances into the CSF. IL-6 is a known marker of inflammation. This is perhaps the mechanism through which they were able to measure glyphosate in the CSF. Since this paper is about a suicidal ingestion it should have no impact on re-registration.	
563	CA 5.9	Seok S-J. et al.	2011	Surfactant volume is an essential element in human toxicity in acute glyphosate herbicide intoxication.	Clinical toxicology (2011), Vol. 49, No. 10, pp. 892	5.4.1 case b) Relevant but supplementary information: Results indicate that treatment of patients with acute glyphosate herbicide intoxication should take into account the volume and not the type of surfactants in herbicide formulations.	The RMS agrees with the applicant's justification.
565	CA 5.9	Shaw G. M. et al.	2014	Early pregnancy agricultural pesticide exposures and risk of gastroschisis among offspring in the San Joaquin Valley of California	Birth Defects Research, Part A: Clinical and Molecular Teratology (2014), Vol. 100, No. 9, pp. 686	5.4.1 case b) Relevant but supplementary information: No new information without clear relevance for the risk assessment.	The RMS agrees with the applicant's justification.
566	CA 5.9	Shaw W.	2017	Elevated Urinary Glyphosate and Clostridia Metabolites With Altered Dopamine Metabolism in Triplets With Autistic Spectrum Disorder or Suspected Seizure Disorder: A Case Study.	Integrative medicine (2017), Vol. 16, No. 1, pp. 50	5.4.1 case b) Relevant but supplementary information: This is a limited case study of 3 individuals, with minimal data on glyphosate exposure.	The RMS agrees with the applicant's justification.
201	CA 5.9.1	Aris A.	2012	Response to comments from Monsanto scientists on our study showing detection of glyphosate and Cry1Ab in blood of women with and without pregnancy	Reproductive Toxicology (2012), Vol. 33, No. 1, pp. 122	5.4.1 case b) Relevant but supplementary information: Correspondence with no new data.	The RMS agrees with the applicant's justification.
281	CA 5.9.1	Dang Q. et al.	2011	Control Effect of Occupational Hazards in Construction Project of Glyphosate Production	Chinese Journal of Public Health Engineering (2011), Vol. 10, no. 2, pp. 111	5.4.1 case b) Relevant but supplementary information: This is a paper describing the evaluation of a glyphosate production facility and a description of how to mitigate risks of exposure to the chemistries involved in glyphosate production.	The RMS agrees with the applicant's justification.
334	CA 5.9.1	Goldstein D. A. et al.	2012	Comment: Aris and Leblanc "Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada".	Reproductive Toxicology (2012), Vol. 33, No. 1, pp. 120	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, comments on Aris et al._2011, Reprod. Toxicol (2011), Vol. 31, pp. 528-533.	The RMS agrees with the applicant's justification.
373	CA 5.9.1	Jomichen J. et al.	2017	Australian work exposures studies: occupational exposure to pesticides.	Occupational and environmental medicine (2017), Vol. 74, No. 1, pp. 46	5.4.1 case b) Relevant but supplementary information: Occupational exposure survey.	The RMS agrees with the applicant's justification.
398	CA 5.9.1	Knudsen L. E. et al.	2017	Biomonitoring of Danish school children and mothers including biomarkers of PBDE and glyphosate.	Reviews on environmental health (2017), Vol. 32, No. 3, pp. 279	5.4.1 case b) Relevant but supplementary information: All glyphosate levels many orders of magnitude lower than the ADI.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
456	CA 5.9.1	Mesnage R. et al.	2012	Glyphosate exposure in a farmer's family.	Journal of Environmental Protection (2012), Vol. 3, No. 9, pp. 1001	5.4.1 case b) Relevant but supplementary information: Glyphosate measured in urine of farmer and family.	The RMS agrees with the applicant's justification.
459	CA 5.9.1	Mills P. J. et al.	2017	Excretion of the Herbicide Glyphosate in Older Adults Between 1993 and 2016.	Journal of the American Medical Association (2017), Vol. 318, No. 16, pp. 1610	5.4.1 case b) Relevant but supplementary information: Not relevant for EU toxicology risk assessment but supplementary information on human exposure.	The RMS agrees with the applicant's justification.
460	CA 5.9.1	Mills P. J. et al.	2018	Excretion of the herbicide glyphosate in older adults between 1993 and 2016 (vol 318, pg 1610, 2017)	Journal of the American Medical Association (2018), Vol. 319, No. 13, pp. 1386	5.4.1 case b) Relevant but supplementary information: Correction to Mills et al._2017, Journal of the American Medical Association (2017), Vol. 318, No. 16, pp. 1610-1611.	The RMS agrees with the applicant's justification.
473	CA 5.9.1	Mueller U. et al.	2012	Comment on "Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada".	Reproductive Toxicology (2012), Vol. 33, No. 3, pp. 401	5.4.1 case b) Relevant but supplementary information: Letter to the Editor, Comments on Aris et al._2011, Reprod. Toxicol (2011), Vol. 31, pp. 528-533.	The RMS agrees with the applicant's justification.
657	CA 5.9.1	Zhang F. et al.	2019	Study on the effect of occupational exposure to glyphosate on blood routine.	Chinese journal of industrial hygiene and occupational diseases (2019), Vol. 37, No. 2, pp. 126	5.4.1 case b) Relevant but supplementary information: No adverse outcome identified.	The RMS agrees with the applicant's justification.
242	CA 5.9.2	Bus J. S.	2015	Analysis of Moms Across America report suggesting bioaccumulation of glyphosate in U.S. mother's breast milk: Implausibility based on inconsistency with available body of glyphosate animal toxicokinetic, human biomonitoring, and physico-chemical data.	Regulatory toxicology and pharmacology (2015), Vol. 73, No. 3, pp. 758	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
249	CA 5.9.2	Campuzano C. et al.	2017	Efectos de la intoxicacion por glifosato en la poblacion agricola: revision de tema	Revista CES Salud Publica (2017), Vol. 8, No. 1, pp. 121	5.4.1 case b) Relevant but supplementary information: This article claims that occupational exposure to glyphosate formulations is associated with multi-organ toxicity via suicidal ingestions and a literature review to support their claim. In suicide attempts, glyphosate based formulations are known to cause caustic injury leading to multi-organ failure. However, occupational exposures do not, nor do they lead to chronic long term effects. The Ag Health Study from 2005 & 2018 demonstrate no evidence of carcinogenicity. The Farm Family Exposure Study shows that there is minimal absorption of glyphosate in the occupational setting.	The RMS agrees with the applicant's justification.
268	CA 5.9.2	Cho Y. S. et al.	2018	The qSOFA Score: A Simple and Accurate Predictor of Outcome in	Basic & clinical pharmacology & toxicology	5.4.1 case b) Relevant but supplementary information: This study is describing the use of a	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				Patients with Glyphosate Herbicide Poisoning.	(2018), Vol. 123, No. 5, pp. 615	scoring system to predict severity of outcome after patients present with a formulated glyphosate overdose. This is meant to guide clinical practice and should not impact re-registration.	
312	CA 5.9.2	Elsner P. et al.	2018	Occupational koebnerization of psoriasis caused by glyphosate.	Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology (2018), Vol. 16, No. 1, pp. 70	5.4.1 case b) Relevant but supplementary information: There is not a mechanism for glyphosate to cause psoriasis, particularly 1 week post exposure.	The RMS agrees with the applicant's justification.
314	CA 5.9.2	Eriguchi M. et al.	2019	Parkinsonism Relating to Intoxication with Glyphosate.	Internal medicine (2019), Vol. 58, No. 13, pp. 1935	5.4.1 case b) Relevant but supplementary information: (Reversible) Parkinsonism in case of acute in-toxication is a well-known effect and not specific for glyphosate.	The RMS agrees with the applicant's justification.
323	CA 5.9.2	Frappart M. et al.	2011	A fatal acute poisoning with glyphosate: importance of gastrointestinal toxicity. Original title: Une intoxication aigue fatale au glyphosate : importance de la toxicite digestive.	Annales francaises d'anesthesie et de reanimation (2011), Vol. 30, No. 11, pp. 852	5.4.1 case b) Relevant but supplementary information: This case report describes caustic injury to the GI tract and multi-organ failure after formulated glyphosate overdose. The clinical course is consistent with previous reports of overdose and should not impact re-registration.	The RMS agrees with the applicant's justification.
335	CA 5.9.2	Goldstein D. A. et al.	2018	Reversible Parkinsonism following glyphosate exposure.	Parkinsonism and Related Disorders (2018), Vol. 56, pp. 107	5.4.1 case b) Relevant but supplementary information: Letter ref to Zheng et al._2018, Parkinsonism Relat Disord. (2018), Vol. 56, pp.108.	The RMS agrees with the applicant's justification.
369	CA 5.9.2	Jayasumana C. et al.	2014	Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka?.	International journal of environmental research and public health (2014), Vol. 11, No. 2, pp. 2125	5.4.1 case b) Relevant but supplementary information: Presents a hypothesis which is not tested, only discussed.	The RMS agrees with the applicant's justification.
370	CA 5.9.2	Jayasumana C. et al.	2015	Simultaneous exposure to multiple heavy metals and glyphosate may contribute to Sri Lankan agricultural nephropathy.	BMC nephrology (2015), Vol. 16, pp. 103	5.4.1 case b) Relevant but supplementary information: Presents a hypothesis which is not tested, only discussed	The RMS agrees with the applicant's justification.
382	CA 5.9.2	Karberg K. et al.	2018	Glyphosate levels in older adults.	JAMA - Journal of the American Medical Association (2018), Vol. 319, No. 13, pp. 1384	5.4.1 case b) Relevant but supplementary information: Medical data which should not impact the re-registration.	The RMS agrees with the applicant's justification.
387	CA 5.9.2	Khot R. et al.	2018	Glyphosate poisoning with acute fulminant hepatic failure.	Asia Pacific Journal of Medical Toxicology (2018), Vol. 7, No. 3, pp. 86	5.4.1 case b) Relevant but supplementary information: glyphosate is not hepatotoxic by any route.	The RMS agrees with the applicant's justification.
408	CA 5.9.2	Langrand J. et al.	2019	Increased severity associated with tallowamine in acute glyphosate poisoning.	Clinical toxicology (2020), Vol. 58, pp. 201	5.4.1 case b) Relevant but supplementary information: In this study, severe respiratory symptoms were also more frequently reported in the	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
						TA group. The surfactant properties of POEA are likely to cause aspiration pneumonitis which is a plausible explanation for the respiratory failure complicating severe GBF poisoning cases.	
422	CA 5.9.2	Lee M-J. et al.	2019	Hemodynamic changes after infusion of intravenous lipid emulsion to treat refractory hypotension caused by glyphosate-surfactant herbicide poisoning A case report.	Medicine (2019), Vol. 98, No. 3, pp. Article No.: e14156	5.4.1 case b) Relevant but supplementary information: This is an article describing the use of lipid emulsion in a suicidal overdose of formulated glyphosate. This has been well described in the literature as a possible intervention in critically ill patients.	The RMS agrees with the applicant's justification.
448	CA 5.9.2	Mariager T. P. et al.	2013	Severe adverse effects related to dermal exposure to a glyphosate-surfactant herbicide.	Clinical toxicology (2013), Vol. 51, No. 2, pp. 111	5.4.1 case b) Relevant but supplementary information: No new effects are discussed in the publication. Adverse effects of formulations in case of dermal exposure are well known. The data should not impact the re-registration.	The RMS agrees with the applicant's justification.
461	CA 5.9.2	Mills P. J. et al.	2018	Erratum: Excretion of the herbicide glyphosate in older adults between 1993 and 2016.	Journal of the American Medical Association (2018), Vol. 319, No. 13, pp. 1386	5.4.1 case b) Relevant but supplementary information: Erratum listing undisclosed conflicts of interest on a previous paper, Mills_2017, Journal of the American Medical Association (2017), Vol. 318, No. 16, pp. 1610-1611.	The RMS agrees with the applicant's justification.
462	CA 5.9.2	Mills P. J. et al.	2020	Glyphosate Excretion is Associated With Steatohepatitis and Advanced Liver Fibrosis in Patients With Fatty Liver Disease.	Clinical gastroenterology and hepatology (2020), Vol. 8, pp. 741	5.4.1 case b) Relevant but supplementary information: No new information without clear relevance for the risk assessment. This paper should not impact the re-registration.	The RMS agrees with the applicant's justification.
463	CA 5.9.2	Mills P. J. et al.	2018	Undisclosed conflicts of interest	Journal of the American Medical Association (2018), Vol. 319, No. 13, pp. 1386	5.4.1 case b) Relevant but supplementary information: Correction to Mills et al._2017, Journal of the American Medical Association 2017, Vol. 318, No. 16, pp. 1610-1611.	The RMS agrees with the applicant's justification.
470	CA 5.9.2	Moon J. M. et al.	2018	Cardiovascular Effects and Fatality May Differ According to the Formulation of Glyphosate Salt Herbicide.	Cardiovascular toxicology (2018), Vol. 18, No. 1, pp. 99	5.4.1 case b) Relevant but supplementary information: Preliminary results without investigation of other factors contributing to such effects.	The RMS agrees with the applicant's justification.
483	CA 5.9.2	Niemann L. et al.	2015	A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers.	Journal fuer Verbraucherschutz und Lebensmittelsicherheit/Journal of Consumer Protection and Food Safety (2015), Vol. 10, No. 1, pp. 3	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
495	CA 5.9.2	Palli E. et al.	2011	Rapture of the large intestine caused by severe oral glyphosate-surfactant intoxication.	The American journal of emergency medicine (2011), Vol. 29, No. 4, pp. 459	5.4.1 case b) Relevant but supplementary information: This article describes corrosive injury to the transverse colon in a suicidal ingestion of formulated glyphosate. This is known to occur in	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
						suicidal overdoses and should not impact re-registration	
538	CA 5.9.2	Rendon-von Osten J. et al.	2017	Glyphosate Residues in Groundwater, Drinking Water and Urine of Subsistence Farmers from Intensive Agriculture Localities: A Survey in Hopelchen, Campeche, Mexico.	International journal of environmental research and public health (2017), Vol. 14, No. 6, pp. E595	5.4.1 case b) Relevant but supplementary information: No new information without clear relevance for the risk assessment.	The RMS agrees with the applicant's justification.
568	CA 5.9.2	Shrestha S. et al.	2018	Incident thyroid disease in female spouses of private pesticide applicators.	Environment International (2018), Vol. 118, pp. 282	5.4.1 case b) Relevant but supplementary information: Very superficial information about exposure to specific pesticides. Limitations in assessment of potential confounding factors. Limitations in exposure and outcome information. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
580	CA 5.9.2	Solomon K. R.	2016	Glyphosate in the general population and in applicators: a critical review of studies on exposures.	Critical reviews in toxicology (2016), Vol. 46, No. sup1, pp. 21	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
658	CA 5.9.2	Zhang F. et al.	2018	Relationships between internal and external exposure indicators of glyphosate in occupational workers.	Journal of Environmental & Occupational Medicine (2018), Vol. 35, No. 11, pp. 990	5.4.1 case b) Relevant but supplementary information: Manufacturing practices in China are not representative of EU manufacturing protocols	The RMS agrees with the applicant's justification.
668	CA 5.9.2	Zheng Q. et al.	2018	Reversible Parkinsonism induced by acute exposure glyphosate.	Parkinsonism & related disorders (2018), Vol. 50, pp. 121	5.4.1 case b) Relevant but supplementary information: Reversible Parkinsonism in case of acute in-toxication is a well-known effect and not specific for glyphosate.	The RMS agrees with the applicant's justification.
669	CA 5.9.2	Zheng Q. et al.	2018	Reply for the comment on "Reversible Parkinsonism induced by acute exposure glyphosate".	Parkinsonism and Related Disorders (2018), Vol. 56, pp. 108	5.4.1 case b) Relevant but supplementary information: Letter to the editor, comments on Goldstein_2018, Parkinsonism Relat Disord. (2018), Vol. 56, pp. 107	The RMS agrees with the applicant's justification.
184	CA 5.9.4	Acquavella J. et al.	2016	Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma.	Critical reviews in toxicology (2016), Vol. 46, No. sup1, pp. 28	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
208	CA 5.9.4	Avgerinou C. et al.	2017	Occupational, dietary, and other risk factors for myelodysplastic syndromes in Western Greece.	Hematology (2017), Vol. 22, No. 7, pp. 419	5.4.1 case b) Relevant but supplementary information: A case-control study with non-blind interviewers results in both potential recall bias and interviewer bias. This publication is considered unreliable.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.9 for an assessment of the study.
210	CA 5.9.4	Avila-Vazquez M. et al.	2015	Cancer and detrimental reproductive effects in an Argentine agricultural community environmentally exposed	Journal of Biological Physics and Chemistry (2015), Vol. 15, No. 3, pp. 97	5.4.1 case b) Relevant but supplementary information: There is no glyphosate use associations quantified, confounded by multiple pesticide uses,	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				to glyphosate. Original Title: Cancer y trastornos reproductivos en una poblacion agricola argentina expuesta a glifosato.		other local industry and local sanitation questions.	
222	CA 5.9.4	Beard J. D. et al.	2014	Pesticide exposure and depression among male private pesticide applicators in the agricultural health study.	Environmental Health Perspectives (2014), Vol. 122, No. 9, pp. 984	5.4.1 case b) Relevant but supplementary information: No statistically significant findings for glyphosate.	The RMS agrees with the applicant's justification.
223	CA 5.9.4	Beard J. D. et al.	2013	Pesticide exposure and self-reported incident depression among wives in the Agricultural Health Study	Environmental Research (2013), Vol. 126, pp. 31	5.4.1 case b) Relevant but supplementary information: No statistically significant findings for glyphosate.	The RMS agrees with the applicant's justification.
243	CA 5.9.4	Caballero M. et al.	2018	Estimated Residential Exposure to Agricultural Chemicals and Premature Mortality by Parkinson's Disease in Washington State.	International journal of environmental research and public health (2018), Vol. 15, No. 12, pp. 1	5.4.1 case b) Relevant but supplementary information: Unproven exposure. Uncertain temporal relationship between purported exposure and the health outcome. Appropriate design would evaluate exposure or non-exposure from Parkinson's diagnosis and compare length of survival by exposure category.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.9 for an assessment of the study.
247	CA 5.9.4	Cai W. et al.	2020	Correlation between CYP1A1 polymorphisms and susceptibility to glyphosate-induced reduction of serum cholinesterase: A case-control study of a Chinese population.	Pesticide biochemistry and physiology (2020), Vol. 162, pp. 23	5.4.1 case b) Relevant but supplementary information: Untenable assumption for the genetic analyses: that ChE depression (viz., case status) is related to glyphosate. Note that ChE depression is not more likely among those with longest glyphosate employment tenure. Adequate description of study population is uncertain. Selection process not clearly described. Adequate description of exposure circumstances is uncertain. Description of workplaces lacking. Subjects could have worked primarily in producing raw materials. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
263	CA 5.9.4	Chang E. T. et al.	2016	Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2016), Vol. 51, No. 6, pp. 402	5.4.1 case b) Relevant but supplementary information: The glyphosate meta-RRs took the results from the available studies at face value. The authors had no way to correct for recall bias, confounding, etc. Therefore, the meta-RRs are in error to the extent that the studies included in the meta-analysis are also in error. Chang and Delzell (2016) are clear on this point in their meta-analysis article. Accordingly glyphosate p-values and confidence intervals for the meta-RRs cannot be taken at face value because they incorporate systematic error or bias. Thus, the argument about	RMS requested a study summary in order to further justify the categorization. Refer to B.6.9 for an assessment of the study.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
						the statistical significance/insignificance of the meta-RR for glyphosate is negated. One cannot calculate a valid p-value when there is uncontrolled systematic error (Greenland S. Randomization, statistics, and causal inference. Epidemiology 1990; 1:421-429).	
272	CA 5.9.4	Conti C. L. et al.	2018	Pesticide exposure, tobacco use, poor self-perceived health and presence of chronic disease are determinants of depressive symptoms among coffee growers from Southeast Brazil	Psychiatry Research (2018), Vol. 260, pp. 187	5.4.1 case b) Relevant but supplementary information: Study is fraught with limitations including very poor statistical analysis. Outcome and exposures essentially concurrent. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
276	CA 5.9.4	Cremonese C. et al.	2017	Occupational exposure to pesticides, reproductive hormone levels and sperm quality in young Brazilian men	Reproductive Toxicology (2017), Vol. 67, pp. 174	5.4.1 case b) Relevant but supplementary information: Due to exposure/outcome temporal ambiguity and failure to control for other exposures in the evaluation of specific exposures. This publication is considered unreliable.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.9 for an assessment of the study.
283	CA 5.9.4	de Araujo J. S A. et al.	2016	Glyphosate and adverse pregnancy outcomes, a systematic review of observational studies.	BMC public health (2016), Vol. 16, pp. 472	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
320	CA 5.9.4	Fluegge K. et al.	2018	Environmental factors influencing the link between childhood ADHD and risk of adult coronary artery disease.	Medical Hypotheses (2018), Vol. 110, pp. 83	5.4.1 case b) Relevant but supplementary information: No new information without clear relevance for the risk assessment.	The RMS agrees with the applicant's justification.
321	CA 5.9.4	Fluegge K. et al.	2016	Glyphosate Use Predicts Healthcare Utilization for ADHD in the Healthcare Cost and Utilization Project net (HCUPnet): A Two-Way Fixed-Effects Analysis.	Polish Journal of Environmental Studies (2016), Vol. 25, No. 4, pp. 1489	5.4.1 case b) Relevant but supplementary information: No new information without clear relevance for the risk assessment.	The RMS agrees with the applicant's justification.
322	CA 5.9.4	Fortes C. et al.	2016	Occupational Exposure to Pesticides With Occupational Sun Exposure Increases the Risk for Cutaneous Melanoma	Journal of occupational and environmental medicine (2016), Vol. 58, No. 4, pp. 370	5.4.1 case b) Relevant but supplementary information: No specific analyses for glyphosate. Interviewers were not blinded. Recall bias may produce spurious positive associations. Confounding not addressed adequately. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
332	CA 5.9.4	Goldner W. S. et al.	2013	Hypothyroidism and Pesticide Use Among Male Private Pesticide Applicators in the Agricultural Health Study	Journal of Occupational and Environmental Medicine (2013), Vol. 55, No. 10, pp. 1171	5.4.1 case b) Relevant but supplementary information: No correlation between effects and glyphosate use.	The RMS agrees with the applicant's justification.
355	CA 5.9.4	Henneberger P. K. et al.	2014	Exacerbation of symptoms in agricultural pesticide applicators with asthma.	International archives of occupational and environmental health (2014), Vol. 87, No. 4, pp. 423	5.4.1 case b) Relevant but supplementary information: No adverse effects correlating with glyphosate use.	The RMS agrees with the applicant's justification.
358	CA 5.9.4	Hoppin J. A. et al.	2017	Pesticides are Associated with	Environmental health	5.4.1 case b) Relevant but supplementary	The RMS agrees with the

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				Allergic and Non-Allergic Wheeze among Male Farmers.	perspectives (2017), Vol. 125, No. 4, pp. 535	information: The exposure and outcome data were concurrent, so a temporal relationship could not be established. The extraordinary number of positive statistically significant findings mitigates against interpreting any one finding as likely to be causal. This publication is considered unreliable.	applicant's justification.
399	CA 5.9.4	Kongtip P. et al.	2019	Thyroid Hormones in Conventional and Organic Farmers in Thailand.	International journal of environmental research and public health (2019), Vol. 16, No. 15, pp. 2704	5.4.1 case b) Relevant but supplementary information: The higher incidence of thyroid disease in women (more numerous in organic farming), no data on the menopausal status of the women (change in thyroid hormones), the collection of data within dairies of the farmers may be incomplete, the exposure of farmers to pesticides prior to the study and prior to starting organic farming, and the results for glyphosate should have been examined for confounding from other pesticides that were correlated with glyphosate use. Moreover, the use rate and bioavailability (Acquavella et al. (2004) Environmental Health Perspectives Vol. 112(3), 321-326; Acquavella et al. (2006) Epidemiology, Vol. 17(1), 69-74) of glyphosate was lower than that of the other pesticides used. Since the determination of serum thyroid hormone levels is key in this study, the methods of analysis should have been better documented. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
413	CA 5.9.4	LaVerda N. L. et al.	2015	Pesticide Exposures and Body Mass Index (BMI) of Pesticide Applicators From the Agricultural Health Study	Journal of Toxicology and Environmental Health, Part A: Current Issues (2015), Vol. 78, No. 20, pp. 1255	5.4.1 case b) Relevant but supplementary information: No relevant endpoint for risk assessment.	The RMS agrees with the applicant's justification.
416	CA 5.9.4	Lebov J. F. et al.	2015	Pesticide exposure and end-stage renal disease risk among wives of pesticide applicators in the Agricultural Health Study	Environmental Research (2015), Vol. 143, No. Part_A, pp. 198	5.4.1 case b) Relevant but supplementary information: Glyphosate was not associated with ESRD, but this study did not have the detail necessary to provide reliable information. Mostly speculative information about exposure to glyphosate and other pesticides. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
426	CA 5.9.4	Leon M. E. et al.	2019	Pesticide use and risk of non-Hodgkin lymphoid malignancies in agricultural cohorts from France, Norway and the USA: a pooled analysis from the AGRICOH	International journal of epidemiology (2019), Vol. 1, No. 48, pp. 1519	5.4.1 case b) Relevant but supplementary information: Due to an error prone exposure methodology and the attendant inability to control confounding. We also note that the results for the Norwegian cohort conflict with the AHS results	RMS requested a study summary in order to further justify the categorization. Refer to B.6.9 for an assessment of the study.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				consortium.		where exposure is determined more specifically and where there is no relationship between glyphosate and DLBCL among individuals in the highest exposed quartile (≥ 108 days). This publication is considered unreliable.	
434	CA 5.9.4	Ling C. et al.	2018	Prenatal Exposure to Ambient Pesticides and Preterm Birth and Term Low Birthweight in Agricultural Regions of California.	Toxics (2018), Vol. 6, No. 3, pp. E41	5.4.1 case b) Relevant but supplementary information: Unproven assumption that residence near land treated with pesticides equates to meaningful exposure. Glyphosate biomonitoring would suggest that is highly implausible. Also, residence on birth certificates is an uncertain indicator of residential proximity to treated land during pregnancy. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
464	CA 5.9.4	Mink P. J. et al.	2011	Epidemiologic studies of glyphosate and non-cancer health outcomes: a review.	Regulatory toxicology and pharmacology (2011), Vol. 61, No. 2, pp. 172	5.4.1 case b) Relevant but supplementary information: This is an epidemiology review article on non-cancer endpoints.	The RMS agrees with the applicant's justification.
465	CA 5.9.4	Mink P. J. et al.	2012	Epidemiologic studies of glyphosate and cancer: a review.	Regulatory toxicology and pharmacology (2012), Vol. 63, No. 3, pp. 440	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
466	CA 5.9.4	Mise M.	2011	Epidemiological study of glyphosate herbicide poisoning.	The Japanese journal of toxicology (2011), Vol. 24, No. 1, pp. 69	5.4.1 case b) Relevant but supplementary information: Epidemiological analysis of acute poisoning cases due to oral ingestion of glyphosate (suicide attempts), clinical symptoms such as metabolic acidosis, hyperkalemia, electrocardiogram abnormalities are known effects and should not impact the re-registration.	The RMS agrees with the applicant's justification.
507	CA 5.9.4	Parks C. G. et al.	2016	Rheumatoid Arthritis in Agricultural Health Study Spouses: Associations with Pesticides and Other Farm Exposures.	Environmental health perspectives (2016), Vol. 124, No. 11, pp. 1728	5.4.1 case b) Relevant but supplementary information: Lack of information about glyphosate frequency of use and timing of use. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
508	CA 5.9.4	Parvez S. et al.	2018	Glyphosate exposure in pregnancy and shortened gestational length: a prospective Indiana birth cohort study	Environmental Health (2018), Vol. 17, pp. 23/1	5.4.1 case b) Relevant but supplementary information: Small study. Uncertain exposure characterization. Premature births were 1 of 5 for those with glyphosate < LOD and 1 of 66 for those with glyphosate > LOD. This suggests no evidence of glyphosate being related to preterm birth. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
512	CA 5.9.4	Perry M. J. et al.	2019	Historical evidence of glyphosate exposure from a US agricultural cohort	Environmental Health (2019), Vol. 18, No. 1, pp. 42	5.4.1 case b) Relevant but supplementary information: The study population, the sampling and the method of analysis along with its validation are not sufficiently documented. This publication is	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
						considered unreliable.	
557	CA 5.9.4	Santos R. et al.	2019	Thyroid and reproductive hormones in relation to pesticide use in an agricultural population in Southern Brazil	Environmental Research (2019), Vol. 173, pp. 221	5.4.1 case b) Relevant but supplementary information: Insufficient information is provided on the biochemical methods used. No detailed description of the analytical methods for the measurement of hormones in serum (using a kit from Roche). This publication is considered unreliable.	The RMS agrees with the applicant's justification.
569	CA 5.9.4	Shrestha S. et al.	2018	Pesticide use and incident hypothyroidism in pesticide applicators in the agricultural health study	Environmental Health Perspectives (2018), Vol. 126, No. 9, pp. 11	5.4.1 case b) Relevant but supplementary information: Self-reported outcomes, lack of biological predicate for many pesticides (including glyphosate), and failure to control for confounding by other pesticides for glyphosate and for other pesticides. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
576	CA 5.9.4	Slager R. E. et al.	2010	Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study	Journal of Toxicology and Environmental Health - Part A: Current Issues (2010), Vol. 73, No. 20, pp. 1382	5.4.1 case b) Relevant but supplementary information: No information on the formulations, farming practice in the given time period has been provided.	The RMS agrees with the applicant's justification.
578	CA 5.9.4	Smpokou E. et al.	2019	Environmental exposures in young adults with declining kidney function in a population at risk of Mesoamerican nephropathy.	Occupational and environmental medicine (2019), Vol. 76, No. 12, pp. 920	5.4.1 case b) Relevant but supplementary information: Too little glyphosate exposure for an informative study. Many confounding exposures. Although this was described as a case control study, the authors did not calculate odds ratios. Evaluation of mean values is not a causal parameter in a case control study. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
629	CA 5.9.4	Wang G. et al.	2011	Parkinsonism after chronic occupational exposure to glyphosate.	Parkinsonism & related disorders (2011), Vol. 17, No. 6, pp. 486	5.4.1 case b) Relevant but supplementary information: Reversible Parkinsonism in case of acute intoxication is a well-known effect and not specific for glyphosate. No clear causal connection of chronic Parkinsonism to glyphosate from the presented results.	The RMS agrees with the applicant's justification.
637	CA 5.9.4	Williams G. M. et al.	2016	A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment.	Critical reviews in toxicology (2016), Vol. 46, No. sup1, pp. 3	5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
655	CA 5.9.4	Zhang C. et al.	2016	Health effect of agricultural pesticide use in China: implications for the development of GM crops	Scientific reports (2016 Vol. 6, pp. 34918	5.4.1 case b) Relevant but supplementary information: Results are likely to be valid for glyphosate under the exposure circumstances of the study, however the study was not appropriately designed for assessment of chronic health effects. In particular, there were short follow-ups and limited exposure histories.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
656	CA 5.9.4	Zhang C. et al.	2018	A comparison of the effects of agricultural pesticide uses on peripheral nerve conduction in China	Scientific Reports (2018), Vol. 8, No. 1, pp. 1	5.4.1 case b) Relevant but supplementary information: Results agree with biological properties of the various pesticides. However, an inappropriate design to study the potentially chronic association between nerve conduction and pesticide exposure. There was short follow-up and limited exposure histories.	The RMS agrees with the applicant's justification.
659	CA 5.9.4	Zhang F. et al.	2017	Study of the effect of occupational exposure to glyphosate on hepatorenal function.	Chinese journal of preventive medicine (2017), Vol. 51, No. 7, pp. 615	5.4.1 case b) Relevant but supplementary information: Poorly described study design, methods, and analysis. This publication is considered unreliable.	The RMS agrees with the applicant's justification.
661	CA 5.9.4	Zhang L. et al.	2019	Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence	Mutation Research, Reviews in Mutation Research (2019), Vol. 781, pp. 186	5.4.1 case b) Relevant but supplementary information: Meta-analyses cannot overcome the limitations of the studies included. This publication is considered unreliable.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.9 for an assessment of the study.
185	CA 5.9.5	Adams R. D. et al.	2013	The NPIS Pesticide Surveillance Project - Eye contact with pesticides: Circumstances of exposure and toxicity.	Clinical Toxicology (2013), Vol. 51, No. 4, pp. 353	5.4.1 case b) Relevant but supplementary information: This is a report describing ocular exposures to pesticides. Formulated glyphosate is expected to cause moderate conjunctivitis & irritation when the eye is exposed due to the surfactant. This should not impact re-registration.	The RMS agrees with the applicant's justification.
231	CA 5.9.5	Bosak A. B. et al.	2014	Clinical presentations with different glyphosate-containing herbicides.	Journal of Medical Toxicology (2014), Vol. 10, No. 1, pp. 72	5.4.1 case b) Relevant but supplementary information: This is a report about multi-organ failure after suicidal ingestion of formulated glyphosate and should not impact re-registration.	The RMS agrees with the applicant's justification.
237	CA 5.9.5	Brunetti R. et al.	2019	Electrocardiographic abnormalities associated with acute glyphosate toxicity.	HeartRhythm Case Rep. (2020), Vol. 6, pp. 63	5.4.1 case b) Relevant but supplementary information: This article claims that dermal exposure to a small amount of glyphosate led to cardiac arrhythmia and claims that the patient developed a Brugada syndrome & long Qt syndrome after exposure. The measured QTC in a wide-complex tracing is uninterpretable. Brugada syndrome is largely due to sodium channel block in cardiac myocytes, LQT syndrome is largely due to potassium channel block in the cardiac myoctyes. Glyphosate does neither. Moreover, glyphosate is not dermally absorbed and multiple GLP studies have shown that glyphosate is not cardiotoxic.	The RMS agrees with the applicant's justification.
244	CA 5.9.5	Caganova B. et al.	2017	Caustic effects of chemicals: risk factors for complications and mortality in acute poisoning	Monatshefte fuer Chemie (2017), Vol. 148, No. 3, pp. 497	5.4.1 case b) Relevant but supplementary information: This article discusses caustic injury in suicide attempts and therefore should not impact	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
						registration decisions.	
245	CA 5.9.5	Caganova B. et al.	2017	Caustic ingestion in the elderly: influence of age on clinical outcome	Molecules (2017), Vol. 22, No. 10, pp. 1726/1	5.4.1 case b) Relevant but supplementary information: This article compares outcomes of caustic ingestions in young to elderly patients and it demonstrates that there is a higher mortality in the older group. Glyphosate is mentioned in a table where there were 9 ingestions with no fatalities in the younger group and 2 fatalities in the elderly. This article discusses suicidal ingestions of caustic substances and should therefore not impact re-registration.	The RMS agrees with the applicant's justification.
254	CA 5.9.5	Carroll R. et al.	2012	Diurnal variation in probability of death following self-poisoning in Sri Lanka--evidence for chronotoxicity in humans.	International journal of epidemiology (2012), Vol. 41, No. 6, pp. 1821	5.4.1 case b) Relevant but supplementary information: This article discusses the concept of chronotoxicity in overdoses. They found no evidence of circadian effects on glyphosate overdoses. This article discusses suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
261	CA 5.9.5	Chan C-W. et al.	2016	Successful Extracorporeal Life Support in a Case of Severe Glyphosate-Surfactant Intoxication.	Critical care medicine (2016), Vol. 44, No. 1, pp. E45	5.4.1 case b) Relevant but supplementary information: This paper looked at the use of ECMO in a critically ill patient after formulated glyphosate product overdose. ECMO is sometime of utility in treating overdose patients. This paper should not impact re-registration.	The RMS agrees with the applicant's justification.
265	CA 5.9.5	Chen H-H. et al.	2013	Spectrum of corrosive esophageal injury after intentional paraquat or glyphosate-surfactant herbicide ingestion.	International journal of general medicine (2013), Vol. 6, pp. 677	5.4.1 case b) Relevant but supplementary information: Ingestions of formulated glyphosate and paraquat are known to cause caustic injury which can result in respiratory and other complications. This paper should not impact the re-registration.	The RMS agrees with the applicant's justification.
267	CA 5.9.5	Cho Y. et al.	2019	Serial measurement of glyphosate blood concentration in a glyphosate potassium herbicide-intoxicated patient: A case report.	The American journal of emergency medicine (2019), Vol. 37, pp 160	5.4.1 case b) Relevant but supplementary information: Measurement of glyphosate blood concentration in an intoxicated patient, no unusal findings for such a case (suicide attempt).	The RMS agrees with the applicant's justification.
269	CA 5.9.5	Cho Y. S. et al.	2019	Use of qSOFA Score in Predicting the Outcomes of Patients With Glyphosate Surfactant Herbicide Poisoning Immediately Upon Arrival at the Emergency Department.	Shock (Augusta, Ga.) (2019), Vol. 51, No. 4, pp. 447	5.4.1 case b) Relevant but supplementary information: This article describes a scoring system that is widely used in intensive care and used to determine the prognosis of patients with a variety of presenting complaints. It is descriptive and helps physicians decide wheter a patient needs early ICU intervention. This article is describing a series of overdoses and should not impact re-registration	The RMS agrees with the applicant's justification.
270	CA 5.9.5	Choi B. et al.	2013	Plasma lactate level may be an insufficient monitoring tool in	Toxicology Letters (2013), Vol. 221, Supp. 1, pp. S66	5.4.1 case b) Relevant but supplementary information: This is a report about measuring IMA	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				critically ill patient: A case of ischemia modified albumin in acute glyphosate poisoning.		rather than lactate as a marker of shock after suicidal ingestion of formulated glyphosate and should not impact re-registration.	
289	CA 5.9.5	De Raadt W. M. et al.	2015	Acute eosinophilic pneumonia associated with glyphosate-surfactant exposure.	Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG (2015), Vol. 32, No. 2, pp. 172	5.4.1 case b) Relevant but supplementary information: This article is a case report of a smoker who developed eosinophilic pneumonia after glyphosate exposure. Glyphosate is not a sensitizer as established by multiple GLP regulatory studies. Nozzle application of formulated glyphosate producess aerosols of between 200-350 microns. In humans, it takes droplets of <100 microns to cause inhalational injury. The claim that formulated glyphosate can cause inhalational injury in a setting where it isn't aspirated is not biologically plausible.	The RMS agrees with the applicant's justification.
298	CA 5.9.5	Deo S. P. et al.	2012	Accidental chemical burns of oral mucosa by herbicide.	Journal of the Nepal Medical Association (2012), Vol. 52, No. 185, pp. 40	5.4.1 case b) Relevant but supplementary information: Large ingestions of formulated glyphosate can often result in caustic injury secondary to the surfactant's detergent actions on the mucous membranes of in people who ingest them. That said, they shouldn't cause microstomia, which tends to result from much more corrosive and scarring chemicals. This should not impact re-registration.	The RMS agrees with the applicant's justification.
326	CA 5.9.5	Garlich F. M. et al.	2014	Hemodialysis clearance of glyphosate following a life-threatening ingestion of glyphosate-surfactant herbicide.	Clinical toxicology (2014), Vol. 52, No. 1, pp. 66	5.4.1 case b) Relevant but supplementary information: This article discusses the sucessful use of haemodialysis in a patient who was critically ill after a formulated glyphosate overdose.	The RMS agrees with the applicant's justification.
331	CA 5.9.5	Gil H-W. et al.	2013	Effect of intravenous lipid emulsion in patients with acute glyphosate intoxication.	Clinical toxicology (2013), Vol. 51, No. 8, pp. 767	5.4.1 case b) Relevant but supplementary information: This paper evaluated the use of lipid therapy to treat formulated glyphosate overdoses. The mortality in these overdoses is usually due to the caustic injury to the mucosa membrane from the surfactant moeity of the product. There is some evidence that lipid emulsion can decrease the toxicity of the surfactant. These are suicidal ingestions and should not impact re-registration.	The RMS agrees with the applicant's justification.
352	CA 5.9.5	Hansen N. B. et al.	2013	Severe toxicity from accidental glyphosate ingestion in a child.	Clinical Toxicology (2013), Vol. 51, No. 4, pp. 354	5.4.1 case b) Relevant but supplementary information: This is a case report of an accidental ingestion of formulated glyphosate resulting in mild corrosive injury to the GI tract in a small child and should not impact re-registration.	The RMS agrees with the applicant's justification.
359	CA 5.9.5	Hour B. T. et al.	2012	Herbicide roundup intoxication:	The American journal of	5.4.1 case b) Relevant but supplementary	The RMS agrees with the

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
				successful treatment with continuous renal replacement therapy.	medicine (2012), Vol. 125, No. 8, pp. 1	information: This article discusses the use of CVVD in formulated glyphosate overdoses and medical management of suicidal ingestions and therefore should not impact registration decisions	applicant's justification.
360	CA 5.9.5	Indirakshi J. et al.	2017	Toxic Epidermal Necrolysis and Acute Kidney Injury due to Glyphosate Ingestion.	Indian journal of critical care medicine (2017), Vol. 21, No. 3, pp. 167	5.4.1 case b) Relevant but supplementary information: Glyphosate based formulations are not known to cause TEN which is a t-cell mediated type IV hypersensitivity reaction. >1% of glyphosate is absorbed through the skin and large ingestions have caustic effects on th GI tract which can result in mult-iorgan failure.	The RMS agrees with the applicant's justification.
363	CA 5.9.5	Iwai K. et al.	2014	Utility of upper gastrointestinal endoscopy for management of patients with roundup poisoning.	Journal of Clinical Toxicology (2014), Vol. 4, No. 6, pp. 1	5.4.1 case b) Relevant but supplementary information: This article discusses the use of endoscopy to treat formulated glyphosate overdose and medical management of suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
374	CA 5.9.5	Jovic-Stosic J. et al.	2013	Lipid emulsion in treatment of cardiovascular collapse in acute poisoning.	Clinical Toxicology (2013), Vol. 51, No. 4, pp. 288.	5.4.1 case b) Relevant but supplementary information: This is a case series that included one patient with a formulated glyphosate overdose and treatment with ILE. This describes medical management of overdoses and should not impact re-registration.	The RMS agrees with the applicant's justification.
375	CA 5.9.5	Jovic-Stosic J. et al.	2016	Intravenous lipid emulsion in treatment of cardiocirculatory disturbances caused by glyphosate-surfactant herbicide poisoning.	Vojnosanitetski pregled (2016), Vol. 73, No. 4, pp. 390	5.4.1 case b) Relevant but supplementary information: Medical case of intentional ingestion. ILE has been proposed as a possible therapy for formulated glyphosate overdoses. As this was a suicide attempt, this should not impact re-registration.	The RMS agrees with the applicant's justification.
376	CA 5.9.5	Jovic-Stosic J. et al.	2016	Antidotal use of intravenous lipid emulsion: 5 years' experience in an intensive care unit.	Clinical Toxicology (2016), Vol. 54, No. 4, pp. 476.	5.4.1 case b) Relevant but supplementary information: This is a report about using ILE to treat overdoses with 1 patient who ingested formulated glyphosate. This paper should not impact re-registration.	The RMS agrees with the applicant's justification.
377	CA 5.9.5	Jyoti W. et al.	2014	Esophageal perforation and death following glyphosate poisoning.	Journal of postgraduate medicine (2014), Vol. 60, No. 3, pp. 346	5.4.1 case b) Relevant but supplementary information: Formulated glyphosate can cause caustic injury to the mucosa membrane after ingestion. The esophagus is especially prone to perforation. Due to the absence of a serosa, the esophagus is notoriously difficult to repair & heal. This is not an unusual feature of caustic injury. As this was a suicide attempt, this should not impact re-registration.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
379	CA 5.9.5	Kamijo Y. et al.	2016	A multicenter retrospective survey of poisoning after ingestion of herbicides containing glyphosate potassium salt or other glyphosate salts in Japan.	Clinical toxicology (2016), Vol. 54, No. 2, pp. 147	5.4.1 case b) Relevant but supplementary information: This article discusses the incidence of hyperkalemia and multi-organ failure after formulated glyphosate ingestions. Neither of these findings are surprising in the setting of potassium salt or surfactant ingestions.	The RMS agrees with the applicant's justification.
380	CA 5.9.5	Kamijo Y. et al.	2012	Glyphosate-surfactant herbicide products containing glyphosate potassium salt can cause fatal hyperkalemia if ingested in massive amounts.	Clinical toxicology (2012), Vol. 50, No. 2, pp. 159	5.4.1 case b) Relevant but supplementary information: This article discusses the fact that certain glyphosate-potassium salt formulations can cause fatal hyperkalemia in overdose. This article discusses a feature of suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
383	CA 5.9.5	Kato Y.	2015	Three cases of an extreme hyperkalemia associated with glyphosate potassium herbicide poisoning	The Japanese journal of toxicology (2015), Vol. 28, No. 4, pp. 368	5.4.1 case b) Relevant but supplementary information: This article describes a case series of three patients who presented with extreme hyperkalemia after suicidal ingestion of formulated glyphosate. This is not unexpected in an ingestion involving glyphosate formulated product with potassium salts and should not affect re-registration.	The RMS agrees with the applicant's justification.
384	CA 5.9.5	Kawagashira Y. et al.	2017	Vasculitic Neuropathy Following Exposure to a Glyphosate-based Herbicide.	Internal medicine (2017), Vol. 56, No. 11, pp. 1431	5.4.1 case b) Relevant but supplementary information: This article discussed the development of painful discoloration of the toes and feet four months after the patient spray applied formulated glyphosate to crops. Interestingly, the patient was taking warfarin therapeutically, which can cause the well-described "purple toe syndrome". There is not a mechanism by which sprayed formulated glyphosate can be absorbed by the skin and directly impact small vasculature or neurons in the feet.	The RMS agrees with the applicant's justification.
390	CA 5.9.5	Kim E. et al.	2016	Patterns of drugs & poisons in southern area of South Korea in 2014.	Forensic Science International (2016), Vol. 269, pp. 50	5.4.1 case b) Relevant but supplementary information: This is an article describing the chemicals / pharmaceuticals that were used in fatal overdoses that were forensically evaluated at the Busan Institute of National Forensic Services. Out of 606 fatalities, agricultural chemicals were involved in 5 and glyphosate was detected in 2 of the cases.	The RMS agrees with the applicant's justification.
391	CA 5.9.5	Kim Y. H. et al.	2014	Heart rate-corrected QT interval predicts mortality in glyphosate-surfactant herbicide-poisoned patients.	The American journal of emergency medicine (2014), Vol. 32, No. 3, pp. 203	5.4.1 case b) Relevant but supplementary information: This article discusses the utility of the QTc interval to predict mortality in suicidal ingestions of glyphosate-based formulation. It is not unexpected for critically ill patients to develop a long QTc.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
392	CA 5.9.5	Kim Y. H. et al.	2016	Prognostic Factors in Emergency Department Patients with Glyphosate Surfactant Intoxication: Point-of-Care Lactate Testing.	Basic & clinical pharmacology & toxicology (2016), Vol. 119, No. 6, pp. 604	5.4.1 case b) Relevant but supplementary information: This study evaluated the use of lactate as a predictor of mortality and found a statistically significant association between a serum lactate of 4.7mmol/L and mortality in formulated glyphosate overdoses. This is not surprising as caustic injury due to detergent-like surfactants will cause cell death and thereby increase lactate levels. This article discusses predictors of mortality in suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
397	CA 5.9.5	Knezevic V. et al.	2012	Early continuous dialysis in acute glyphosate-surfactant poisoning	Srpski arhiv za celokupno lekarstvo (2012), Vol. 140, No. 9-10, pp. 648	5.4.1 case b) Relevant but supplementary information: Glyphosate based formulations can cause renal injury in overdose, and the K+ formulations may result in hyperkalemia. It is therefore reasonable to start hemodialysis or hemofiltration in critically ill patients with kidney failure or hyperkalemia. As this was a suicide attempt, this should not impact re-registration.	The RMS agrees with the applicant's justification.
419	CA 5.9.5	Lee B. K. et al.	2012	Continuous renal replacement therapy in a patient with cardiac arrest after glyphosate-surfactant herbicide poisoning.	Hong Kong Journal of Emergency Medicine (2012), Vol. 19, No. 3, pp. 214	5.4.1 case b) Relevant but supplementary information: This is a report about multi-organ failure and the use of CVVHD after suicidal ingestion of formulated glyphosate and should not impact re-registration.	The RMS agrees with the applicant's justification.
420	CA 5.9.5	Lee D. H. et al.	2017	Severe glyphosate-surfactant intoxication: Successful treatment with continuous renal replacement therapy.	Hong Kong Journal of Emergency Medicine (2017), Vol. 24, No. 1, pp. 40	5.4.1 case b) Relevant but supplementary information: This is a report about multi-organ failure and the use of dialysis after suicidal ingestion of formulated glyphosate and should not impact re-registration.	The RMS agrees with the applicant's justification.
423	CA 5.9.5	Lee W. J. et al.	2012	Incidence of acute occupational pesticide poisoning among male farmers in South Korea	American Journal of Industrial Medicine (2012), Vol. 55, No. 9, pp. 799	5.4.1 case b) Relevant but supplementary information: This article describes a survey performed to assess the incidence of pesticide poisoning in S. Korea. The researchers interviewed 1958 farmers and asked if they exhibited any of the 21 following symptoms: nausea, vomiting, diarrhoea, sore throat, runny nose, dyspnea, headache, dizziness, hyperactivity, profuse sweating, blurred vision, paresthesia, slurred speech, paralysis, chest pain, syncope, muscle weakness,skin irritation, eye irritation, lacrimation , and fatigue. Based on these answers they categorized the farmers into mild, moderate or severe occupational exposure categories.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
						There were 26 formulated glyphosate exposures 17 mild and 9 moderate, with zero fatalities. Based on this self-reported exposure data, they made the following claim: "acute occupational pesticide poisoning was 24.7 (95% CI 22.1–27.2) per 100 male farmers, which corresponds to 209,512 cases across South Korea in 2010." This report supports the data that occupational exposure to glyphosate based products have a very low toxicity profile.	
435	CA 5.9.5	Ling S. L. et al.	2018	Workplace chemical and toxin exposures reported to a Poisons Information Centre: A diverse range causing variable morbidity.	European Journal of Emergency Medicine (2018), Vol. 25, No. 2, pp. 134	5.4.1 case b) Relevant but supplementary information: This article describes the characteristics of toxin/chemical exposures reported to an Austrailian poison center. Glyphosate is mentioned in 1 table only with no description of effects.	The RMS agrees with the applicant's justification.
440	CA 5.9.5	Luo W. et al.	2019	Surgical treatment of pyloric stenosis caused by glyphosate poisoning: A case report.	Medicine (2019), Vol. 98, No. 30, pp. e16590	5.4.1 case b) Relevant but supplementary information: This article describes a case report of gastric ulceration and swelling causing pyloric obstruction in a patient who ingested formulated glyphosate. This is not unexpected as formulations contain surfactants which can cause caustic injury to the GI tract with suicidal ingestions. This should not impact re-registration.	The RMS agrees with the applicant's justification.
441	CA 5.9.5	Mahendrakar K. et al.	2014	Glyphosate surfactant herbicide poisoning and management.	Indian journal of critical care medicine (2014), Vol. 18, No. 5, pp. 328	5.4.1 case b) Relevant but supplementary information: ILE has been proposed as a possible therapy for formulated glyphosate overdoses.	The RMS agrees with the applicant's justification.
467	CA 5.9.5	Mohamed F. et al.	2016	Mechanism-specific injury biomarkers predict nephrotoxicity early following glyphosate surfactant herbicide (GPSH) poisoning.	Toxicology letters (2016), Vol. 258, pp. 1	5.4.1 case b) Relevant but supplementary information: This article discusses the use of biomarkers to predict kidney injury in formulated glyphosate overdose and predictors of nephrotoxicity in suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
471	CA 5.9.5	Moon J. M. et al.	2016	The characteristics of emergency department presentations related to acute herbicide or insecticide poisoning in South Korea between 2011 and 2014.	Journal of toxicology and environmental health. Part A (2016), Vol. 79, No. 11, pp. 466	5.4.1 case b) Relevant but supplementary information: This study showed a decrease in the case fatality rate of suicidal pesticide ingestions between 2011-2014 in South Korea. This clearly demonstrates that herbicides with a lower acute toxicity profile are associated with lower mortality in suicidal ingestions.	The RMS agrees with the applicant's justification.
477	CA 5.9.5	Nakae H. et al.	2015	Paralytic ileus induced by glyphosate intoxication successfully treated using Kampo medicine.	Acute medicine & surgery (2015), Vol. 2, No. 3, pp. 214	5.4.1 case b) Relevant but supplementary information: This article describes alternative medicine therapies that were used to treat a Japanese woman with a paralytic ileus after glyphosate	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
						ingestion. It is not uncommon for patients in a critical care setting to develop an ileus. These tend to resolve on their own without intervention. I cannot be commented on whether this intervention increases GI motility.	
478	CA 5.9.5	Nakayama T. et al.	2019	Renal cortical hypoperfusion caused by glyphosate-surfactant herbicide.	Clinical and experimental nephrology (2019), Vol. 23, No. 6, pp. 865	5.4.1 case b) Relevant but supplementary information: This was a suicidal ingestion of formulated glyphosate that resulted in poor renal perfusion & multiorgan failure. Since this was a suicidal ingestion, the outcome is not unexpected and should not impact the re-egistration.	The RMS agrees with the applicant's justification.
488	CA 5.9.5	Ordonez J. et al.	2013	Non-Ethanol hyperlipasemia in toxicology consultation.	Clinical Toxicology (2013), Vol. 51, No. 7, pp. 703	5.4.1 case b) Relevant but supplementary information: This is a case series looking at the toxic causes of pancreatitis in overdose patients. One of whom had ingested formulated glyphosate. This should not imapct re-registration.	The RMS agrees with the applicant's justification.
491	CA 5.9.5	Ozaki T. et al.	2017	Severe Glyphosate-Surfactant Intoxication Successfully Treated With Continuous Hemodiafiltration and Direct Hemoperfusion: Case Report.	Therapeutic apheresis and dialysis (2017), Vol. 21, No. 3, pp. 296	5.4.1 case b) Relevant but supplementary information: This article discusses the use of haemodialysis and haemofiltration in formulated glyphosate overdoses.This article discusses medical management of suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
506	CA 5.9.5	Park S. et al.	2016	Concurrent Hemoperfusion and Hemodialysis in Patients with Acute Pesticide Intoxication.	Blood Purification (2016), Vol. 42, No. 4, pp. 329	5.4.1 case b) Relevant but supplementary information: This article describes the use of hemodialysis and hemoperfusion in pesticide overdoses. Out of 383 pesticide ingestions 110 were glyphosate formulations. Of the 80 deaths reported 12 of them were glyphosate. This article is describing a possibly beneficial modality of treating severe pesticide overdose and should not impact re-registration.	The RMS agrees with the applicant's justification.
514	CA 5.9.5	Picetti E. et al.	2017	Glyphosate ingestion causing multiple organ failure: A near-fatal case report.	Acta Biomedica (2017), Vol. 88, No. 4, pp. 533	5.4.1 case b) Relevant but supplementary information: This is a report about multi-organ failure after suicidal ingestion of formulated glyphosate and should not impact re-registration.	The RMS agrees with the applicant's justification.
517	CA 5.9.5	Planche V. et al.	2019	Acute toxic limbic encephalopathy following glyphosate intoxication.	Neurology (2019), Vol. 92, No. 11, pp. 534	5.4.1 case b) Relevant but supplementary information: This article discusses the neurologic sequelae of glyphosate ingestion. Glyphosate cannot cross the blood brain barrier. It is not neurotoxic.	The RMS agrees with the applicant's justification.
545	CA 5.9.5	Rother H.	2012	Improving poisoning diagnosis and surveillance of street pesticides	SAMJ (2012), Vol. 102, No. 6, Special Iss., pp. 485	5.4.1 case b) Relevant but supplementary information: No new information included.	The RMS agrees with the applicant's justification.
586	CA 5.9.5	Sribanditmongkol	2012	Pathological and toxicological	The American journal of	5.4.1 case b) Relevant but supplementary	The RMS agrees with the

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
		P. et al.		findings in glyphosate-surfactant herbicide fatality: a case report.	forensic medicine and pathology (2012), Vol. 33, No. 3, pp. 234	information: Description of a case of poisoning / suicidal ingestions of formulated glyphosate cause caustic injury, it is not unusual to find ulceration and haemorrhage of the GI tract in lethal ingestions.	applicant's justification.
597	CA 5.9.5	Takeuchi I. et al.	2019	Decrease in Butyrylcholinesterase Accompanied by Intermediate-like Syndrome after Massive Ingestion of a Glyphosate-surfactant.	Internal medicine (2019), Vol. 15; No. 58, pp. 3057	5.4.1 case b) Relevant but supplementary information: Description of a poisoning case related to a surfactant, symptoms are not unusual.	The RMS agrees with the applicant's justification.
604	CA 5.9.5	Thakur D. S. et al.	2014	Glyphosate poisoning with acute pulmonary edema.	Toxicology international (2014), Vol. 21, No. 3, pp. 328	5.4.1 case b) Relevant but supplementary information: This is a case report of the clinical manifestations of glyphosate-based herbicide ingestions and discusses predictors of mortality in suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
619	CA 5.9.5	Varnai V. M. et al.	2013	Report of the poison control centre for the period 1 January - 31 December 2012. Original title: Izvjesce centra za kontrolu otrovanja za razdoblje od 1. Sijecnja do 31. Prosinca 2012.	Arhiv za Higijenu Rada i Toksikologiju (2013), Vol. 64, No. 1, pp. 183	5.4.1 case b) Relevant but supplementary information: This is a report from the Croatian Poison Center documenting types of exposure reported in 2012. Of the 134 calls regarding pesticide exposure, 84 demonstrated "effects" with 9 described as "serious". Glyphosate was listed as one of the pesticides demonstrating a serious effect. There were no other details provided and there were no fatalities as a result of pesticide exposure.	The RMS agrees with the applicant's justification.
621	CA 5.9.5	Veale D. J. H. et al.	2013	Toxicovigilance I: a survey of acute poisonings in South Africa based on tygerberg poison information centre data	SAMJ (2013), Vol. 103, No. 5, pp. 293	5.4.1 case b) Relevant but supplementary information: This article summarises the chemicals used in South Africa for suicide. Glyphosate is only mentioned in a table in the article as being involved in 23 cases over a 1 year period accounting for 0.9% of the overall cases reported.	The RMS agrees with the applicant's justification.
625	CA 5.9.5	Vidyadhara et al.	2014	Atypical presentation of glyphosate poisoning.	Indian Journal of Critical Care Medicine (2014), Vol. 18, Suppl. 1, pp. S36.	5.4.1 case b) Relevant but supplementary information: This is a report about multiorgan failure after suicidal ingestion of formulated glyphosate and should not impact re-registration.	The RMS agrees with the applicant's justification.
628	CA 5.9.5	Wang D. et al.	2019	Successful extracorporeal membrane oxygenation support for severe acute diquat and glyphosate poisoning: A case report.	Medicine (2019), Vol. 98, No. 6., pp. e14414	5.4.1 case b) Relevant but supplementary information: This article describes using ECMO to manage a patient with multiorgan failure after formulated glyphosate and diquat ingestion. Since this is describing medical management of suicidal overdoses, it should not impact reregistration	The RMS agrees with the applicant's justification.
640	CA 5.9.5	Wu C. J. et al.	2015	PiCCO interpretation for acute glyphosate intoxication with shock: Favors cardiogenic origin.	Clinical Toxicology (2015), Vol. 53, No. 4, pp. 329	5.4.1 case b) Relevant but supplementary information: This is a report regarding multiorgan failure following suicidal ingestion of formulated	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
						glyphosate and should not impact re-registration.	
641	CA 5.9.5	Wu I-L. et al.	2015	Glyphosate intoxication resulting in ventricular dysrhythmias and cardiogenic shock.	Clinical Toxicology (2015), Vol. 53, No. 4, pp. 329	5.4.1 case b) Relevant but supplementary information: This is a report regarding multiorgan failure and use of ECMO following suicidal ingestion of formulated glyphosate and should not impact re-registration.	The RMS agrees with the applicant's justification.
642	CA 5.9.5	Wu M-H. et al.	2015	Successful treatment with hemodialysis for acute renal failure after glyphosate poisoning: A case report.	Clinical Toxicology (2015), Vol. 53, No. 4, pp. 330	5.4.1 case b) Relevant but supplementary information: This is a report about renal failure and haemodialysis after suicidal ingestion of formulated glyphosate and should not impact re-registration.	The RMS agrees with the applicant's justification.
643	CA 5.9.5	Wunnapuk K. et al.	2014	Use of a glyphosate-based herbicide-induced nephrotoxicity model to investigate a panel of kidney injury biomarkers.	Toxicology letters (2014), Vol. 225, No. 1, pp. 192	5.4.1 case b) Relevant but supplementary information: Formulation tested in vivo (Concentrate Roundup Weedkiller, 360 g/L isopropylamine salt, Australia) at high acute doses of 250 - 2500 mg/kg.	The RMS agrees with the applicant's justification.
650	CA 5.9.5	You M-J. et al.	2015	Clostridium tertium bacteremia in a patient with glyphosate ingestion.	The American journal of case reports (2015), Vol. 16, pp. 4	5.4.1 case b) Relevant but supplementary information: This article discussed the use of haemodialysis in the management of hyperkalemia and metabolic acidosis after formulated glyphosate overdose. Haemodialysis is often used to manage refractory hyperkalemia and acidosis. This article discusses medical management of suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
652	CA 5.9.5	You Y. et al.	2012	Effect of intravenous fat emulsion therapy on glyphosate-surfactant-induced cardiovascular collapse.	The American journal of emergency medicine (2012), Vol. 30, No. 9, pp. 2097.e1	5.4.1 case b) Relevant but supplementary information: This article is discussing the efficacy of intravenous fat emulsion as therapy for formulated glyphosate overdose. This report contributes to the evidence that intravenous fat emulsion may be a useful treatment for glyphosate overdose as it may limit the toxicity associated with large surfactant ingestions. There are no RCTs for this as it is a suicidal overdose situation.	The RMS agrees with the applicant's justification.
653	CA 5.9.5	Yu G. C. et al.	2017	The clinical analytics of 10 patients with acute glyphosate poisoning	Chinese journal of industrial hygiene and occupational diseases (2017), Vol. 35, No. 5, pp. 382	5.4.1 case b) Relevant but supplementary information: This is a case study describing the clinical course of 10 patients who drank formulated glyphosate. There were no long-term sequelae of ingestion, and all 10 patients survived. These were suicdal ingestions and should not impact re-registration.	The RMS agrees with the applicant's justification.

No	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
670	CA 5.9.5	Zouaoui K. et al.	2013	Determination of glyphosate and AMPA in blood and urine from humans: about 13 cases of acute intoxication.	Forensic science international (2013), Vol. 226, No. 1-3, pp. E20	5.4.1 case b) Relevant but supplementary information: This report demonstrates a link between higher blood and urine concentrations with formulated glyphosate overdoses and a poorer outcome. This is unsurprising as it reflects that patients drank a larger volume. Larger volumes of formulated product are associated with more toxicity due to the caustic nature of the surfactant, not the amount of active ingredient. All of the laboratory parameters are expected in critically ill patients. As these were suicidal ingestions, this paper should not impact re-registration.	The RMS agrees with the applicant's justification.
671	CA 5.9.5	Zyoud S. H. et al.	2017	Global research production in glyphosate intoxication from 1978 to 2015: A bibliometric analysis.	Human & experimental toxicology (2017), Vol. 36, No. 10, pp. 997	5.4.1 case b) Relevant but supplementary information: This article analyzes the reports of increase in glyphosate intoxications from the early 1970s-2016. Given the increase in use over the same time period it is not surprising that there has been a increase in reporting. This should not impact re-registration.	The RMS agrees with the applicant's justification.

Table B.6.10-2b: Overview of relevant but supplementary (category B) articles after detailed assessment – ***top up literature search 2020***

The applicant is requested to submit a summary and an evaluation of the following Category B (supplementary) studies as AGG disagrees that the studies should be considered as supplementary only. An evaluation and conclusion of these studies should be provided by GRG.

- No. 37 Tang et al., 2020; Glyphosate exposure induces inflammatory responses in the small intestine and alters gut microbial composition in rats.
- No 13 Donato et al., 2020; Exposure to glyphosate and risk of non-Hodgkin lymphoma and multiple myeloma: an updated meta-analysis.

Submission Number	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
43	CA 5.1	Zoller O. *et al.*	2020	Urine glyphosate level as a quantitative biomarker of oral exposure.	International journal of hygiene and environmental health, (2020) Vol. 228, Art. No.113526	5.4.1 case b) relevant but supplementary information: This was a study with human volunteers. The trial was designed to ensure comparable exposure levels to glyphosate among participants who consumed diets with low content of glyphosate residue during the 4-day trial, except for the single meal with targeted amount of glyphosate and AMPA corresponding to an intake of 196.8 µg of glyphosate and 1.67 µg of AMPA. Only urine was collected and analysed for glyphosate and AMPA. Blood and faeces were not collected and/or analysed. This goal of the study was to estimate oral glyphosate intake using urinary biomonitoring data. However, the authors recognised that the determination of blood concentrations is necessary to improve human bioavailability data. Comparison of urinary data in humans in this study with those measured in the rat studies suggest that the systemic availability is much lower in humans than in rats and could be about 20-fold lower. However, in the absence of a mass balance, and a very low recovery of glyphosate and AMPA, the data should be considered unreliable. Given the knowledge that orally dosed glyphosate is mostly excreted via the faeces, an appropriate study design to address mass balance could easily have been implemented to make this a robust and informative investigation. Low recovery rates of glyphosate and AMPA suggest a very large capacity for errors. The study design is inadequate to confirm reliability of the findings. The lack of mass balance of analytes, despite common knowledge that orally dosed glyphosate is mostly excreted in faeces, is disappointing, given the ease with which a mass balance could have been assessed. **The article was downgraded to Category B due to its non-reliability.**	The RMS agrees with the applicant's justification.
37	CA 5.3	Tang Q. *et al.*	2020	Glyphosate exposure induces inflammatory responses in the small intestine and alters gut microbial composition in	Environmental pollution, (2020) Vol. 261, Art. No. 114129	5.4.1 case b) relevant but supplementary information: The rats were gavaged with 0, 5, 50, and 500 mg/kg of body weight glyphosate for 35 continuous days. The different segments of the small intestine were sampled to measure indicators of oxidative stress, ion concentrations and inflammatory responses, and fresh feces were collected for microbiota analysis. The investigation of potential effects on the gut microbiome of	In this study inflammatory responses in the small intestines in rats are studied after 35 day-

Submission Number	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
				rats.		ruminants is not a data requirement for the approval of pesticides and suitable test protocols to assess these effects are not specified in the form of official guidance documents. No GLP status stated, no HCD provided and no purity of glyphosate stated. Fundamental parameters to understand animal health and toxicology endpoints are not reported. Therefore the context of the study results can not be interpreted with any degree of certainity. **The article is not reliable.**	repeated oral gavage with 0, 5, 50, and 500 mg/kg bw. The study was performed in rats and not in ruminants. **Although several limitations/deviations are identified, this in vivo study should be presented in the RAR as similar deviations were also noted for other open literature studies.**
16	CA 5.5	Berry C.	2020	Glyphosate and cancer: the importance of the whole picture.	Pest management science, (2020); doi: 10.1002/ps.5834; Online ahead of print.	5.4.1 case b) relevant but supplementary information: The author is providing a general picture of the carcinogenic and genotoxic profile of Glyphosate by commenting the different studies available and the different conclusions made by IARC and Regulatory authorities. There is no evidence in the animals studies to support the IARC conclusion that glyphosate is a probable human carcinogen. The article does not provide any new information.	The RMS agrees with the applicant's justification.
24	CA 5.5	Jeon S. *et al.*	2020	Glyphosate influences cell proliferation in vitro.	Frontiers in Life Science, (2020) Vol. 13, No. 1, pp. 54-65	5.4.1 case b) relevant but supplementary information: Glyphosate was tested in vitro at a range of doses to investigate its effects on cell growth and proliferation in human cells. In conclusion, Glyphosate increases the rate of cell growth in human embryonic kidney 293 (HEK293) cells. Glyphosate promotes cell proliferation by activating gene expression of cell cycle regulators in humans in vitro. Useful information but not altering risk assessment and data requirement and difficult to be used because no HCD provided. No positive control were used, no statistics methods were described. Furthermore, no OECD guideline followed, no GLP status stated. **The article is not reliable.**	The RMS agrees with the applicant's justification.
14	CA 5.6	Ait-Bali Y. *et al.*	2020	Pre- and postnatal exposure to glyphosate-based herbicide causes behavioral and cognitive impairments in adult mice: evidence of cortical ad hippocampal dysfunction.	Archives of toxicology, (2020) Vol. 94, No. 5, pp. 1703-1723	5.4.1 case b) relevant but supplementary information: In vivo study on pre and post natal effects of Roundup on swiss mice at 2 different doses only, no OECD guideline followed, no GLP status stated, no HCD provided. Oral gavage dosing of formulated product is not relevant to real life exposure scenarios. Environmental fate and metabolism for glyphosate active ingredient versus sufractants are different, and oral co-exposures to mammals at the excessively high doses tested in this case are considered irrelevant to human health risk assessment. In addition, insufficient information is provided to determine which formulation was tested and whether it is the glyphosate EU representative formulation.	Refer to B.6.7.3.4 for an assessment of the study.
17	CA 5.6	Cai W. *et*	2020	Low-dose Roundup	Environmental	5.4.1 case b) relevant but supplementary information: The effects of Roundup at 3	Refer to B.6.6.3.2 for

Submission Number	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
		al.		induces developmental toxicity in bovine preimplantation embryos in vitro.	science and pollution research international, (2020) Vol. 27, No. 14, pp. 16451-16459	doses was investigated on the bovine preimplantation embryo. Direct dosing of formulated product to fertilized embryos in vitro is not relevant to real life exposure scenarios. Environmental fate, metabolism and pharmaco-kinetics for glyphosate active ingredient versus sufractants are very different, and oral co-exposures to mammals at the excessively high doses tested in this case are considered irrelevant to livestock and human health risk assessments. In addition, insufficient information is provided to determine which formulation was tested and whether it is the glyphosate EU representative formulation. No OECD guideline followed, no GLP status stated, no HCD provided and no positive control.	an assessment of the study.
29	CA 5.7	Neto de Silva K. *et al.*	2020	Glyphosate-based herbicide impairs energy metabolism and increases autophagy in C6 astroglioma cell line.	Journal of toxicology and environmental health. Part A, (2020) Vol. 83, No. 4, pp. 153-167	5.4.1 case b) relevant but supplementary information: In vitro study on the effects of micromolar concentrations of a glyphosate-based herbicide on energy metabolism and mitochondrial mass in astroglioma cell line exposed for 24 h to the herbicide at 3 concentrations below 160 µM. Insufficient information provided to identify which formulation was tested. No positive control was used, no statistics methods were described, no OECD guideline followed, no GLP status stated, no HCD provided. In addition, astrocytes in real life are not co-exposed to the combination of glyphosate + surfactant formulants, based on their very different environmental fates and pharmaco-kinetics.	The RMS agrees with the applicant's justification.
26	CA 5.8.2	Levine S. L. *et al.*	2020	Review and Analysis of the Potential for Glyphosate to Interact with the Estrogen, Androgen, and Thyroid Pathways.	Pest management science, (2020), DOI 10.1002/ps.5983	5.4.1 case b) relevant but supplementary information: A systematic literature review was performed including US EPA EDSP Tier 1 battery assessment, guideline regulatory studies, ESDP including 5 in vitro and 6 in vivo assays to evaluate the EAT pathways. From the available literature, it was concluded that glyphosate does not have an endocrine disrupting potential through estrogenic, androgenic or steroidogenic activity. The review includes relevant literature which has been used for the ED assessment during the current submission process. It can therefore serve as supporting information, however as a review it does not provide new primary data or alter the risk assessment. Therefore, the review has been classified as a relevant but supplementary only (EFSA 2092 GD Point 5.4.1 category B).	The RMS agrees with the applicant's justification.
30	CA 5.8.2	Parks C. G. *et al.*	2019	Lifetime pesticide use and antinuclear antibodies in male farmers from the agricultural health study	Frontiers in Immunology (2019) Vol. 10, Art. No. 1476	5.4.1 case b) relevant but supplementary information: The development of systemic autoimmunity in response to pesticide exposure was investigated in a retrospective study farmers. Serum antinuclear autoantibodies were measured by immunofluorescence on Hep-2 cells in 668 male farmers. The effect of lifetime use of 46 pesticides (glyphosate among them) on ANA were investigated. The results for glyphosate use demonstrate no increase the risk of farmers developing systemic autoimmunity. This information is useful in a weight of evidence assessment for the measured endpoint, which, however, is not a critical endpoint identified for human health risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
13	CA 5.9	Abdel-Halim K. Y. *et al.*	2019	Glyphosate and pendimethalin in breast milk samples from Egyptian rural areas: a	International Journal of Advanced Research, (2019)	5.4.1 case b) relevant but supplementary information: This article claims that glyphosate was detected in breast milk. There are several technical issues with this study: 1st: The solubility of glyphosate in toluene is reported as only 36 PPM. The highest sample values the paper claims is just under 30 PPM. So if we had roughly 30	RMS requested a study summary in order to further justify the categorization.

Submission Number	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
				pilot study for infant's risk assessment	Vol. 7, No. 9, pp. 991-1002	PPM of glyphosate in milk and took 5 mL for analysis then the toluene would have to be capable of solubilizing 150 PPM of glyphosate! 2nd: And this is a key issue – the HPLC method lists an excitation wavelength that is higher than the emission wavelength! There are several studies evaluating whether glyphosate is detectable in cows milk. A study in human breast milk also was conducted and concluded that glyphosate was not detectable. References: 1. Michelle K McGuire, Mark A McGuire, William J Price, Bahman Shafii, Janae M Carrothers, Kimberly A Lackey, Daniel A Goldstein, Pamela K Jensen, John L Vicini, Glyphosate and aminomethylphosphonic acid are not detectable in human milk, The American Journal of Clinical Nutrition, Volume 103, Issue 5, May 2016, Pages 1285–1290, https://doi.org/10.3945/ajcn.115.126854 2. EFSA. (2018). National summary reports on pesticide residue analysis performed in 2016. EFSA Journal, 16(7), 5348. https://doi.org/org/10.2903/sp.efsa.2018.EN-1454 3. EFSA. (2019). The 2017 European Union report on pesticide residues in food. EFSA Journal, 17(6), 5743. https://doi.org/ 10.2903/j.efsa.2019.5743 4. EFSA. (2020). The 2018 European Union report on pesticide residues in food. EFSA Journal, 18(4), e06057. https://doi.org/10.2903/j.efsa.2020.6057 5. FDA. (2018). Pesticide residue monitoring program. Fiscal year 2016 pesticide report. FDA. https://www.fda.gov/Food/FoodborneIllnessContaminants/Pesticides/ucm618247.htm 6. FDA. (2019). Pesticide residue monitoring program fiscal year 2017 pesticide report. https://www.fda.gov/food/pesticides/pesticide-residue-monitoring-2017-report-and-data 7. Ehling, S., & Reddy, T. M. (2015). Analysis of glyphosate and aminomethylphosphonic acid in nutritional ingredients and milk by derivatization with fluorenylmethyloxycarbonyl chloride and liquid chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry, 63(48), 10562-10568. https://doi.org/10.1021/acs.jafc.5b04453 8. NZ Ministry for Primary Industries. (2012). Dairy national chemical contaminants programme - raw milk result summary 2011/12. Retrieved 12/15/2015 from http://www.foodsafety.govt.nz/elibrary/industry/dairy-nccp-results-summary.pdf 9. Steinborn, A., Alder, L., Michalski, B., Zomer, P., Bendig, P., Martinez, S. A., Mol, H. G., Class, T. J., & Costa Pinheiro, N. (2016). Determination of glyphosate levels in breast milk samples from Germany by LC-MS/MS and GC-MS/MS. Journal of Agricultural and Food Chemistry, 64(6), 1414-1421., https://doi.org/10.1021/acs.jafc.5b05852 10. von Soosten, D., Meyer, U., Hüther, L., Dänicke, S., Lahrssen-Wiederholt, M., Schafft, H., Spolders, M., & Breves, G. (2016). Excretion pathways and ruminal disappearance of glyphosate and its degradation product aminomethylphosphonic acid	Refer to B.6.6.3.2 for an assessment of the study.

Submission Number	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
						in dairy cows. Journal of Dairy Science, 99(7), 5318-5324. https://doi.org/10.3168/jds.2015-10585 11. Zhao, J., Pacenka, S., Wu, J., Richards, B. K., Steenhuis, T., Simpson, K., & Hay, A. G. (2018). Detection of glyphosate residues in companion animal feeds. Environmental Pollution, 243(Pt B), 1113-1118. https://doi.org/10.1016/j.envpol.2018.08.100 **The article is not reliable.**	
18	CA 5.9	Donato F. *et al.*	2020	Exposure to glyphosate and risk of non-Hodgkin lymphoma and multiple myeloma: an updated meta-analysis.	La Medicina del lavoro, (2020) Vol. 111, No. 1, pp. 63-73	5.4.1 case b) relevant but supplementary information: The publication is considered not reliable because there is nothing that has been done in this (or other) meta-analysis to address recall bias, selection bias, and failure to control for confounding factors in the NHL case-control studies. **The article was downgraded to Category B due to its non-reliability.**	Although in case-control studies recall or selection bias may occur and correction for confounding is limited, it is not a reason for downgrading this publication as supplementary only. The meta-analysis might provide useful information as several studies are taken into account which are considered reliable or reliable with restrictions in the current assessment. **An evaluation and conclusion of these studies should be provided by the applicant.**
19	CA 5.9	Eddleston M.	2020	Poisoning by pesticides.	Medicine, (2020) Vol. 48, No. 3, pp. 214-217	5.4.1 case b) relevant but supplementary information: This is a review article discussing clinical features and management of pesticide overdoses. The article comments that glyphosate has much lower toxicity in acute overdose than older pesticides and discusses the use of supportive care in these overdoses. Since this describes the management of suicidal overdoses it should not impact the risk assessment / re-registration.	The RMS agrees with the applicant's justification.
31	CA 5.9	Rajput R. *et al.*	2019	Haemodialysis as an imperative treating modality in severe	Journal, Indian Academy of Clinical	5.4.1 case b) relevant but supplementary information: This is a case report of a patient who developed hyperkalaemia, renal failure and pulmonary edema after a suicidal ingestion of formulated glyphosate. These clinical features are common with large	The RMS agrees with the applicant's justification.

Submission Number	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
				glyphosate-surfactant poisoning.	Medicine, (2019) Vol. 20, No. 3-4, pp. 224-226	ingestions. Hemodialysis is standard of care in cases such as these. This report raises no new clinical features regarding this type of overdose and should not impact risk assessment / re-registration.	
32	CA 5.9	Ren Y. *et al.*	2020	Cases report of gastrointestinal hemorrhage caused by glyphosate herbicides.	Acta Medica Mediterranea, (2020) Vol. 36, No. 3, pp. 1611-1614	5.4.1 case b) relevant but supplementary information: This article describes two patients with GI hemhorrage after formulated glyphosate ingestion. According to the history, the first patient drank excessive amounts of ethanol for a long time, which in and of itself can contribute to GI ulceration and bleeding. He also ingested triazolone. This patient's course appears to be atypical as the patient appears to have been stable on admission, was in the hospital for weeks, underwent multiple endoscopic procedures for 2 weeks after ingestion and later developed significant GI bleeding necessitating a gastrectomy. In formulated glyphosate overdoses, corrosive injury to the GI tract occurs early due to the surfactant. The second patient in this report also presented with corrosive injury to the GI tract which is not unexpected. Since this paper describes suicidal ingestions it should not impact the risk assessment / re-registration.	The RMS agrees with the applicant's justification.
36	CA 5.9	Soukup S. T. *et al.*	2020	Glyphosate and AMPA levels in human urine samples and their correlation with food consumption: results of the cross-sectional KarMeN study in Germany.	Archives of toxicology, (2020) Vol. 94, No. 5, pp. 1575-1584	5.4.1 case b) relevant but supplementary information: The authors calculated the intake of glyphosate and AMPA based on urinary concentrations and checked this value against the EU acceptable daily intake (ADI) value for glyphosate. The exposure to glyphosate and AMPA was found to be very low. Quantifiable levels of glyphosate and/or AMPA was detected in 8.3% (25 out or 301) of the participants with the highest reported value (0.63 µg/kg BW) being 0.13% of the ADI. 24-hr urine samples were collected from 301 adults for analysis of glyphosate. The study subjects were recruited to be healthy and not taking medications. The glyphosate exposures as a percent of the ADI were calculated. However, unlike previous studies, this calculation was not derived using assumptions for body weight or volume of urine. Rather, ADIs were calculated for each study subject using their own body weight and 24-hr urine excretion. Samples were analyzed using an LC-MS/MS that was modified by the procedure of Jensen *et al.* (1), and 66.5% had neither no detectable glyphosate nor AMPA in urine. Glyphosate and/or AMPA was quantifiable detected in 8.3% of participants with a maximum glyphosate exposure of 0.63 µg/kg BW, which was 0.13% of the ADI. The maximum intake of AMPA + glyphosate corresponded to 0.16% of the ADI. This study also used 24-hr dietary recalls and did rank-order correlations to estimate food sources of glyphosate and AMPA. This was done based solely of the amount of food and not glyphosate content of the food. Nevertheless, they found that consumption of pulses and mushrooms were correlated with glyphosate and AMPA in urine, respectively. Absorbed glyphosate is not metabolized in the body suggesting that ingestion of AMPA per se, not glyphosate, was responsible for urinary AMPA. As a result of their study, the authors concluded that "based on the current risk assessment of glyphosate by EFSA, such exposure levels are not expected to pose any risk to human health. The detected associations with consuming certain foods are in line with reports on glyphosate and AMPA residues in food."	The RMS agrees with the applicant's justification.

Submission Number	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
						References 1. Jensen, P. K., Wujcik, C. E., McGuire, M. K., and McGuire, M. A. (2016) Validation of reliable and selective methods for direct determination of glyphosate and aminomethylphosphonic acid in milk and urine using LC-MS/MS. Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes 51, 254-259	
38	CA 5.9	Uengchuen K. *et al.*	2020	Health risk assessment on the glyphosate exposure of knapsack sprayers.	Indian Journal of Public Health Research and Development, (2020) Vol. 11, No. 3, pp. 2088-2093	5.4.1 case b) relevant but supplementary information: This article describes an assessment tool designed by the researchers to evaluate level of exposure based on PPE use, self-reported symptoms 6 months after use and frequency of use. They found that most farmers used PPE and had minimal symptoms such as burning eyes which may be due to the surfactant in formulations. There were no severe symptoms and no description of long-term outcomes. This descriptive article describes self-reported non-specific symptoms (nausea, headache, rash, burning eyes) in glyphosate users and should not affect the risk assesment / re-registration.	The RMS agrees with the applicant's justification.
39	CA 5.9	Yang F. *et al.*	2020	Acute obstructive fibrinous laryngotracheobronchitis induced by severe glyphosate surfactant intoxication: A case report.	World journal of emergency medicine, (2020) Vol. 11, No. 2, pp. 125-126	5.4.1 case b) relevant but supplementary information: This is a case report describing a patient who developed fibrinous tracheobronchitis after a suicide attempt with formulated glyphosate. Since the surfactant can cause corrosive injury and the patient had evidence of aspiration, this would be a possible side effect. Since this reflects a suicidal ingestion, it should not impact the risk assessment / re-registration.	The RMS agrees with the applicant's justification.
41	CA 5.9	Zhang F. *et al.*	2020	Concentration Distribution and Analysis of Urinary Glyphosate and Its Metabolites in Occupationally Exposed Workers in Eastern China.	International journal of environmental research and public health, (2020) Vol. 17, No. 8, Art. No. 2943	5.4.1 case b) relevant but supplementary information: This study followed workers who were occupationally exposed to glyphosate in a manufacturing facility. They measured ambient air concentrations and then measured urinary concentrations of glyphosate and AMPA and found that the detection rates of glyphosate (>0.020mg/L) and AMPA (>0.010mg/L) were 86.6% (116/134) and 81.3% (109/134), respectively. The median values were 0.292 mg/L and 0.068 mg/L for urinary glyphosate and AMPA. There was variability in exposure based on where the worker was physically in the plant. This study was looking at biomarkers for exposure and makes no health claims regarding thise exposures.	The RMS agrees with the applicant's justification.

Category C

Table B.6.10-3a: Overview of articles of unclear relevance (Category C) after detailed assessment – *initial literature search*

No	Data requirement (indicated by the corresponding CA / CP data point No.)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
673	CA 5.3	Aitbali Y. et al.	2018	Glyphosate based- herbicide exposure affects gut microbiota, anxiety and depression-like behaviors in mice.	Neurotoxicology and teratology (2018), Vol. 67, pp. 44	5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. This study uses Roundup administered at half of or at the NOAEL concentration via a stomach tube. The surfactant is irritating and any negative results are not surprising. The acidic effect of glyphosate is also a concern.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.
675	CA 5.8	Bote K. et al.	2019	Minimum Inhibitory Concentration of Glyphosate and of a Glyphosate-Containing Herbicide Formulation for Escherichia coli Isolates - Differences Between Pathogenicand Non-pathogenic Isolates and Between Host Species.	Frontiers in microbiology (2019), Vol. 10, pp. 932	5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. The study uses a system designed to measure antibiotic MICs that are usually done by culturing bacteria in a specific media for antibiotiic diffusion in ug/ml range. Instead the paperlooks at glyphosate in mg/ml range following MIC procedures. There is no justification for the dose, which should be at about 100000X lower dose. Most gut microbes microbes are anaerobes.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.
680	CA 5.8	Kruger M. et al.	2013	Glyphosate suppresses the antagonistic effect of Enterococcus spp. on Clostridium botulinum.	Anaerobe (2013), Vol. 20, pp. 74	5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. Moreover, the doses used in this study are not justified and are unrealistically high. Cultures are batch culture and it is unclear if conditions are to get values in growing phase. Comparisons between glyphosate and Roundup are completely different so they cannot be compared.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.
679	CA 5.8.2	Good P.	2018	Evidence the U.S. autism epidemic initiated by acetaminophen (Tylenol) is aggravated by oral antibiotic amoxicillin/clavulanate (Augmentin) and now exponentially by herbicide glyphosate (Roundup).	Clinical nutrition ESPEN (2018), Vol. 23, pp. 171	5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. This paper contains no new data. It uses computer algorithms to make associations that are not proved. It claims that glyphosate impacts methionine and tryptophan and ignores that these amino acids are not only essential for the human diet but that microbially derived amino acids are only available via coprophagy.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.

No	Data requirement (indicated by the corresponding CA / CP data point No.)	Author(s)	Year	Title	Source	Justification applicant	RMS comments
681	CA 5.8.2	Lozano V. L. et al.	2018	Sex-dependent impact of Roundup on the rat gut microbiome.	Toxicology reports (2018), Vol. 5, pp. 96	5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. This study has a number of issues related to design: Rats are at the end of their life when feces were sampled. It is not clear of feces were sampled pre- or post mortem. Results are confounded by advanced age or even tumor status of these rats, predominantly mammary. The smaller than expected number of phyla may be related to age of the rats. Short-term responses are not surprising: cells in direct contact with a substance in a test tube (liquid medium) will respond differently than cells exposed to that same substance within their natural environment. So in vitro data usually show cells have a greater sensitivity to the substance than in vivo data. And within the intestinal environment there is much to dilute, diminish or mask the substance's effect. This diminished effect in vivo has been documented repeatedly for a large number of test substances.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.
682	CA 5.8.2	Mao Q. et al.	2018	The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome.	Environmental Health (2018), Vol. 29, No. 17, pp 50	5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. In this publication there was no clinical evidence of alterations in activity or behavior in pups. Body weight, water and feed consumption both in dams and pups were no different across the groups. Litter sizes were fully comparable among groups. To identify changes in microbes with multiple analyses in groups of animals is not unexpected and not necessarily indicative of a specific effect of the active substance. Changes within all rats due to maturation are greater than the differences between treatment groups. Moreover there are several points limiting the significance of the results: 1) information to calculate dose is not in the paper and seems intentional, 2) ADI is not the same as exposure which averages 1% of the ADI, and clinical signs were by definition not observed at the NOAEL which is 100-fold greater than the ADI. Animals in these toxicity studies had gut microbes, 3) Claims of exposure via milk are unfounded. The statistical analysis results in some differences but they do not put these changes into the context of whether they are normal.	RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.

Table B.6.10-3b: Overview of articles of unclear relevance (Category C) after detailed assessment – ***top up literature search 2020***

Submission Number	Data requirement (indicated by the corresponding CA / CP data point number)	Author(s)	Year	Title	Source	Justification	RMS comments
None	None	None	None	None	None	None	None

Non-relevant articles

Table B.6.10-4a: Overview of articles excluded after detailed assessment (i.e. not relevant) – *initial literature search*

No	Technical section	Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria)	RMS comments
1251	Toxicology and metabolism	Abarikwu S. O. et al.	2015	Combined effects of repeated administration of Bretmont Wipeout (glyphosate) and Ultrazin (atrazine) on testosterone, oxidative stress and sperm quality of Wistar rats.	Toxicology mechanisms and methods (2015), Vol. 25, No. 1, pp. 70-80	Formulation provided to Wistar rats via oral gavage in corn oil.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary. The study summary submitted was considered as too brief and a more detailed summary, covering both ED and toxicity to reproduction, is listed as a data gap.
1252	Toxicology and metabolism	Abass K. et al.	2012	Characterization of human cytochrome P450 induction by pesticides	Toxicology (2012), Vol. 294, No. 1, pp. 17-26	No significant glyphosate related effects.	The RMS agrees with the applicant's justification.
1253	Toxicology and metabolism	Abass K. et al.	2013	The inhibition of major human hepatic cytochrome P450 enzymes by 18 pesticides: Comparison of the N-in-one and single substrate approaches	Toxicology In Vitro (2013), Vol. 27, No. 5, pp. 1584-1588	Glyphosate not mentioned in the paper.	The RMS agrees with the applicant's justification.
1254	Toxicology and metabolism	Aboukila R. S. et al.	2014	Cytogenetic study on the effect of bentazon and glyphosate herbicide on mice.	Alexandria Journal of Veterinary Sciences (2014), Vol. 41, pp. 95-101	This publication is considered not relevant because a glyphosate formulation (Glalica) was used instead of glyphosate and the route of administration was intraperitoneal injection which is an inappropriate route of administration for the occupational and food risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1255	Toxicology and metabolism	Addae J. I. et al.	2011	Effects of AMPA and clomethiazole on spreading depression cycles in the rat neocortex in vivo	European Journal of Pharmacology (2011), Vol. No. 1-3, pp. 41-46	The article is investigating AMPA Receptor with drugs applied i.p. and topically.	The RMS agrees with the applicant's justification.
1256	Toxicology and metabolism	Alarcon R. et al.	2019	Neonatal exposure to a glyphosate-based herbicide alters the histofunctional differentiation of the ovaries and uterus in lambs.	Molecular and cellular endocrinology (2019), Vol. 482, pp. 45-56	Formulation tested (Roundup Full II, Argos SRL, Santa Fe, Argentina; 54 g/100 mL glyphosate)	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1257	Toxicology and metabolism	Altamirano G. A. et al.	2018	Postnatal exposure to a glyphosate-based herbicide modifies mammary gland growth and development in Wistar male rats.	Food and chemical toxicology (2018), Vol. 118, pp. 111-118	Formulation tested (Roundup FULL II, potassium salt; 54% a.e.)	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1258	Toxicology and	Aminov A. I. et al.	2013	Effect of the herbicide Roundup on the activity of Glycosidases of	Inland Water Biology (2013), Vol. 6, No. 4, pp. 351-356	Formulation tested in vitro (Roundup, produced and packaged by ZAO Avgust, Russia; 36%	The RMS agrees with the applicant's justification.

	metabolism			invertebrates and juvenile fish.		glyphosate).	
1259	Toxicology and metabolism	Anakwue R.	2019	Cardiotoxicity of Pesticides: Are Africans at Risk?	Cardiovascular toxicology (2019), Vol. 19, No. 2, pp. 95-104	Review article with no new data.	The RMS agrees with the applicant's justification.
1260	Toxicology and metabolism	Anifandis G. et al.	2017	The In Vitro Impact of the Herbicide Roundup on Human Sperm Motility and Sperm Mitochondria.	Toxics (2017), Vol. 6, No. 1, pp. 2	Formulation tested in vitro (Roundup, not characterized).	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1261	Toxicology and metabolism	Williams, G. M. et al.	2018	Corrigendum to: A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment (Critical Reviews in Toxicology, (2016), 46, sup1, (3-20), 10.1080/10408444.2016.1214677).	Critical Reviews in Toxicology (2018), Vol. 48, No. 10, pp 907-908	Corrigendum to paper updating acknowledgements and author conflicts of interest.	The RMS agrees with the applicant's justification.
1262	Toxicology and metabolism	Mesnage R. et al.	2017	Erratum to: Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure (Environmental Health: A Global Access Science Source (2015) 14:70 DOI: 10 1186/s12940-015-0056-1).	Environmental Health (2017), Vol. 16, No. 1, pp. 28	This is erratum to Mesnage et al., Environmental health (2015), Vol. 14, article No. 70.	The RMS agrees with the applicant's justification.
1263	Toxicology and metabolism	Anon.	2013	Pesticide Exposure in Children (vol 130, pg e1757, 2012).	Pediatrics (2013), Vol. 131, No. 5, pp. 1013	No glyphosate data generated/presented.	The RMS agrees with the applicant's justification.
1264	Toxicology and metabolism	Antoniou M. N. et al.	2019	Glyphosate does not substitute for glycine in proteins of actively dividing mammalian cells.	BMC research notes (2019), Vol. 12, No. 1, pp. 494	This publication is found not relevant because the end-point investigated (substitution of glycine by glyphosate in protein synthesis) is not appropriate for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1265	Toxicology and metabolism	Aroonvilairat S. et al.	2015	Effect of pesticide exposure on immunological, hematological and biochemical parameters in Thai orchid farmers-a cross-sectional study	International Journal of Environmental Research and Public Health (2015), Vol. 12, No. 6, pp. 5846-5861	This is a general pesticides paper and not specific to glyphosate.	The RMS agrees with the applicant's justification.
1266	Toxicology and metabolism	Asita A. O. et al.	2012	Cytotoxicity and genotoxicity of some agropesticides used in Southern Africa	Journal of Toxicology and Environmental Health Sciences (2012), Vol. 4, No. 10, pp. 175-184	Formulation tested in vitro (Wipe-out, Kombat (Pty) Ltd, South Africa; 360 g/L glyphosate). Tested a plant species with a herbicide for adverse end-points; not relevant to human health end-points.	The RMS agrees with the applicant's justification.
1267	Toxicology and metabolism	Astiz M. et al.	2012	The oxidative damage and inflammation caused by pesticides are reverted by lipoic acid in rat brain.	Neurochemistry international (2012), Vol. 61, No. 7, pp. 1231-41	In vivo administration via intraperitoneal injection which is not a relevant exposure route for EU glyphosate renewal.	The RMS agrees with the applicant's justification.
1268	Toxicology and metabolism	Astiz M. et al.	2013	Pesticide-induced decrease in rat testicular steroidogenesis is differentially prevented by lipoate	Ecotoxicology and environmental safety (2013), Vol. 91, pp. 129-38	In vivo administration via intraperitoneal injection which is not a relevant exposure route for EU glyphosate renewal.	The RMS agrees with the applicant's justification.

				and tocopherol.			
1269	Toxicology and metabolism	Avdatek F. et al.	2018	Ameliorative effect of resveratrol on testicular oxidative stress, spermatological parameters and DNA damage in glyphosate-based herbicide-exposed rats.	Andrologia (2018), Vol. 50, No. 7, pp. e13036	Glyphosate based formulation tested (Knockdown 48 SL) which is not comparable to the EU renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1270	Toxicology and metabolism	Avdatek F. et al.	2018	Protective effect of N-acetylcysteine on testicular oxidative damage, spermatological parameters and DNA damage in glyphosate-based herbicide-exposed rats.	Kocatepe Veterinary Journal (2018), Vol. 11, No. 3, pp. 292-300	Formulation tested (Knockdown 48 SL, Turkey) which is not comparable to the EU renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1271	Toxicology and metabolism	Avila-Vazquez M. et al.	2018	Environmental exposure to glyphosate and reproductive health impacts in agricultural population of Argentina.	Journal of Environmental Protection (2018), Vol. 9, No. 3, pp. 241-253	This publication is considered not relevant for the risk assessment of glyphosate because the general population followed was exposed to multiple environmental factors making it impossible to establish a causal relationship between exposure to glyphosate and reproductive disorders.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1272	Toxicology and metabolism	Ayanda O. I. et al.	2012	Histopathological examination of the liver and gills of Clarias gariepinus treated with glyphosate.	Environmental Research Journal (2012), Vol. 6, No. 3, pp. 228-234	Formulation tested in aquatic species (Roundup 480 mg/L isopropanol salt; 360 g/L a.e.). Effects clearly attributable to surfactant.	The RMS agrees with the applicant's justification.
1273	Toxicology and metabolism	Babic Z. et al.	2019	Report of the Poison Control Centre for the period from 1 January to 31 December 2018; Original title: Izvjesce Centra za kontrolu otrovanja za razdoblje od 1. sijecnja do 31. prosinca 2018	Arhiv Za Higijenu Rada i Toksikologiju (2019), Vol. 70, No. 1, pp. 69-73	Glyphosate based herbicide mentioned once and no glyphosate specific data included in the study.	The RMS agrees with the applicant's justification.
1274	Toxicology and metabolism	Bader M. A. et al.	2015	Effect of quercetin against Roundup and/or fluoride induced biochemical alterations and lipid peroxidation in rats	International Journal of Pharmaceutical Sciences Review and Research (2015), Vol. 34, No. 2, pp. 168-175	Excessively high 28-day repeat dose at 500 mg/kg/day glyphosate based herbicide and is therefore not comparable to the EU glyphosate renewal.	The RMS agrees with the applicant's justification.
1275	Toxicology and metabolism	Bali Y. A. et al.	2019	Learning and memory impairments associated to acetylcholinesterase inhibition and oxidative stress following glyphosate based-herbicide exposure in mice.	Toxicology (2019), Vol. 415, pp. 18-25	Formulation tested (Roundup herbicide (glyphosate concentration 360 g/l IPA salt, Monsanto) which contains a surfactant not present in the representative glyphosate used in the EU renewal process.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1276	Toxicology and metabolism	Bates N. et al.	2013	Glyphosate toxicity in animals.	Clinical Toxicology (2013), Vol. 51, No. 10, pp. 1243	Correspondence adds no new data on human health end-points.	The RMS agrees with the applicant's justification.
1277	Toxicology and metabolism	Beecham J. E. et al.	2015	The possible link between autism and glyphosate acting as glycine mimetic - a review of evidence from the literature with analysis	Journal of Molecular and Genetic Medicine (2015), Vol. 9, No. 4, pp. 1000197/1-1000197/16	This publication is considered not relevant for glyphosate risk assessment because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
1278	Toxicology and metabolism	Bellantuono V. et al.	2014	Pesticides alter ion transport across frog (Pelophylax kl. esculentus) skin	Chemistry in ecology (2014), Vol. 30, No. 7, pp. 602-610	End-point not relevant to human health risk assessment in the EU renewal.	The RMS agrees with the applicant's justification.
1279	Toxicology and	Benitez Leite S. et al.	2019	DNA damage induced by exposure to pesticides in children of rural	Indian journal of medical research (2019), Vol. 150, No.	No evaluation of the glyphosate used as part of the study. Study provides a comparison of	The RMS agrees with the applicant's justification.

	metabolism			areas in Paraguay	3, pp. 290-296	children living near transgenic soybean fields to a control group near crops managed with biological controls.	
1280	Toxicology and metabolism	Beranger R. et al.	2018	Multiple pesticide analysis in hair samples of pregnant French women: Results from the ELFE national birth cohort.	Environment International (2018), Vol. 120, pp. 43-53	No data presented on glyphosate, therefore not relevant for the EU renewal.	The RMS agrees with the applicant's justification.
1281	Toxicology and metabolism	Bernieri T. et al.	2019	Occupational exposure to pesticides and thyroid function in Brazilian soybean farmers.	Chemosphere (2019), Vol. 218, pp. 425-429	General pesticide exposures, not glyphosate specific, therefore not relevant for the EU renewal.	The RMS agrees with the applicant's justification.
1282	Toxicology and metabolism	Bernieri T. et al.	2019	Effect of pesticide exposure on total antioxidant capacity and biochemical parameters in Brazilian soybean farmers	Drug and Chemical Toxicology (2019), Ahead of Print	General pesticide exposure biomonitoring study, not glyphosate specific and therefore not relevant for the EU renewal.	The RMS agrees with the applicant's justification.
1283	Toxicology and metabolism	Bhardwaj J. K. et al.	2019	Effective attenuation of glyphosate-induced oxidative stress and granulosa cell apoptosis by vitamins C and E in caprines.	Molecular reproduction and development (2019), Vol. 86, No. 1, pp. 42-52	Glyphosate based herbicide tested with in vitro test system. As this formulation is not the representative formulation used in the EU renewal process, it is not relevant.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1284	Toxicology and metabolism	Buralli R. J. et al.	2018	Respiratory condition of family farmers exposed to pesticides in the state of Rio de Janeiro, Brazil.	International Journal of Environmental Research and Public Health (2018), Vol. 15, No. 6, pp. 1203	General pesticide exposures, not glyphosate specific, therefore not relevant for the EU renewal.	The RMS agrees with the applicant's justification.
1285	Toxicology and metabolism	Burella P. M. et al.	2017	Evaluation of Stage-Dependent Genotoxic Effect of Roundup(®) (Glyphosate) on Caiman latirostris Embryos.	Archives of environmental contamination and toxicology (2017), Vol. 72, No. 1, pp. 50-57	Glyphosate based herbicide tested on reptiles. End-point and species not relevant to EU annex I renewal.	The RMS agrees with the applicant's justification.
1286	Toxicology and metabolism	Camacho A. et al.	2017	The health consequences of aerial spraying illicit crops: The case of Colombia.	Journal of health economics (2017), Vol. 54, pp. 147-160	This publication is considered not relevant for the risk assessment of glyphosate because it is too general and no specific epidemiological method was followed to establish an association between the application of glyphosate and disease outcome.	The RMS agrees with the applicant's justification.
1287	Toxicology and metabolism	Caramello C. S. et al.	2017	Evaluation of herbicide glyphosate effects in the fish Prochilodus lineatus using chromosome aberration test.	Revista Veterinaria (2017), Vol. 28, No. 1, pp. 65-68	Formulation tested (Roundup Full II), not representative for the renewal.	The RMS agrees with the applicant's justification.
1288	Toxicology and metabolism	Cassault-Meyer E. et al.	2014	An acute exposure to glyphosate-based herbicide alters aromatase levels in testis and sperm nuclear quality.	Environmental toxicology and pharmacology (2014), Vol. 38, No. 1, pp. 131-40	This publication is considered not relevant for the risk assessment of glyphosate because a glyphosate formulation (Roundup Grand Travaux Plus) was tested instead of glyphosate.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1289	Toxicology and metabolism	Castelani P. et al.	2013	Novel adjuvants for high load glyphosate formulations	SOFW Journal (2013), Vol. 139, No. 6, pp. 30-34,36	Formulation chemistry paper and therefore not relevant to the EU renewal of glyphosate.	The RMS agrees with the applicant's justification.
1290	Toxicology and metabolism	Cattani D. et al.	2017	Developmental exposure to glyphosate-based herbicide and depressive-like behavior in adult	Toxicology (2017), Vol. 387, pp. 67-80	Formulation tested (Roundup Original, Brazil, 360 g/L glyphosate), not-representative for the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1

				offspring: Implication of glutamate excitotoxicity and oxidative stress.			of Volume 3 CA B.6.6 for RMS comments and summary.
1291	Toxicology and metabolism	Cattani D. et al.	2014	Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity.	Toxicology (2014), Vol. 320, pp. 34-45	Formulation tested (Roundup Original, Brazil, 360 g/L glyphosate), not-representative for the renewal.	The RMS agrees with the applicant's justification.
1292	Toxicology and metabolism	Cattelan M. D. P. et al.	2018	Occupational exposure to pesticides in family agriculture and the oxidative, biochemical and hematological profile in this agricultural model	Life Sciences (2018), Vol. 203, pp. 177-183	General pesticide exposures, not glyphosate specific and thus not relevant to the EU renewal of glyphosate.	The RMS agrees with the applicant's justification.
1293	Toxicology and metabolism	Cavusoglu K. et al.	2011	Protective effect of Ginkgo biloba L. leaf extract against glyphosate toxicity in Swiss albino mice.	Journal of medicinal food (2011), Vol. 14, No. 10, pp. 1263-72	Single dose administration intraperitoneally as well as the protective effect of a Ginkgo biloba extract. This is not representative of glyphosate exposure and therefore not relevant to the renewal.	The RMS agrees with the applicant's justification.
1294	Toxicology and metabolism	Cermak A. M. M. et al.	2018	Redox imbalance caused by pesticides: a review of OPENTOX-related research.	Arhiv za higijenu rada i toksikologiju (2018), Vol. 69, No. 2, pp. 126-134	A review article of in vitro studies with no new data provided.	The RMS agrees with the applicant's justification.
1295	Toxicology and metabolism	Chaufan G. et al.	2014	Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: differences with its active ingredient.	International journal of toxicology (2014), Vol. 33, No. 1, pp. 29-38	No effects for glyphosate and AMPA, only with formulation tested in an in vitro system. Data not biologicaly relevant to the renewal.	The RMS agrees with the applicant's justification.
1296	Toxicology and metabolism	Chlopecka M. et al.	2014	Glyphosate affects the spontaneous motoric activity of intestine at very low doses - in vitro study.	Pesticide biochemistry and physiology (2014), Vol. 113, pp. 25-30	A novel ex-vivo model not relevant to the EU renewal of glyphosate.	The RMS agrees with the applicant's justification.
1297	Toxicology and metabolism	Chlopecka M. et al.	2017	The effect of glyphosate-based herbicide Roundup and its co-formulant, POEA, on the motoric activity of rat intestine - In vitro study.	Environmental toxicology and pharmacology (2017), Vol. 49, pp. 156-162	Formulation and mixtures of glyphosate and surfactant tested in vitro (Roundup ULTRA 170 SL; 170 g isopropylamine salt/L). Data not biologically relevant to the renewal.	The RMS agrees with the applicant's justification.
1298	Toxicology and metabolism	Clair E. et al.	2012	A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels.	Toxicology in vitro (2012), Vol. 26, No. 2, pp. 269-79	This publication is considered not relevant for the risk assessment of glyphosate as a glyphosate formulation (Roundup Bioforce) was used instead of glyphosate for in vitro testing.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1299	Toxicology and metabolism	Clark P. A. et al.	2016	Chronic kidney disease in Nicaraguan sugarcane workers: A historical, medical, environmental analysis and ethical analysis.	Internet Journal of Third World Medicine (2016), Vol. 12, No. 1	This publication is considered not relevant for glyphosate risk assessment because no systematic epidemiological approach was followed. Similarly figures for workers were not reported, nor were exposure patterns observed.	The RMS agrees with the applicant's justification.
1300	Toxicology and metabolism	Clausing P.	2017	Cancer risk by glyphosate: The "Weight of Evidence Approach" of BfR. Krebsgefahr durch Glyphosat: Der "Weight of Evidence Approach"	Umweltmedizin Hygiene Arbeitsmedizin (2017), Vol. 22, No. 1, pp. 27-34	This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work.	The RMS agrees with the applicant's justification.

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1301	Toxicology and metabolism	Clausing P. et al.	2018	Pesticides and public health: an analysis of the regulatory approach to assessing the carcinogenicity of glyphosate in the European Union.	Journal of epidemiology and community health (2018), Vol. 72, No. 8, pp. 668-672	No new data, a commentary article therefore not relevant for the renewal of glyphosate.	The RMS agrees with the applicant's justification.
1302	Toxicology and metabolism	Coalova I. et al.	2014	Influence of the spray adjuvant on the toxicity effects of a glyphosate formulation.	Toxicology in vitro (2014), Vol. 28, No. 7, pp. 1306-11	The formulation tested in vitro (Atanor, Argentina; 48% glyphosate isopropylamine salt) is not therepresentative formulation for the renewal.	The RMS agrees with the applicant's justification.
1303	Toxicology and metabolism	Coon E. A. et al.	2019	Conjugal multiple system atrophy: Chance, shared risk factors, or evidence of transmissibility?.	Parkinsonism and Related Disorders (2019), Vol. 67, pp. 10-13	Glyphosate use is one of many potential environmental factors considered as a cause for multiple system atrophy, with no specific information provided.	The RMS agrees with the applicant's justification.
1304	Toxicology and metabolism	Cortinovis C. et al.	2015	Glyphosate-surfactant herbicide poisoning in domestic animals: an epidemiological survey.	The Veterinary record (2015), Vol. 176, No. 16, pp. 413	Acute poisoning in animals, not relevant fort he renewal.	The RMS agrees with the applicant's justification.
1305	Toxicology and metabolism	Coullery R. P. et al.	2016	Neuronal development and axon growth are altered by glyphosate through a WNT non-canonical signaling pathway.	Neurotoxicology (2016), Vol. 52, pp. 150-61	High in vitro doses >10 mM of glyphosate, therefore not representative of use/exposure and not relevant for the renewal.	The RMS agrees with the applicant's justification.
1306	Toxicology and metabolism	Dar M. A.. et al.	2015	Single and interactive toxic potential of Roundup and ammonium nitrate on Haemato-biochemical parameters in wistar rats	Journal of Cell and Tissue Research (2015), Vol. 15, No. 3, pp. 5295-5299	High dose of Glyphosate based herbicide administered to rats in drinking water. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1307	Toxicology and metabolism	Dar M. A. et al.	2018	Sub-acute oral toxicity of Roundup® and ammonium nitrate with special reference to oxidative stress indices in wistar rats.	Indian Journal of Animal Research (2018), Vol. 52, No. 3, pp. 405-408	Formulation tested Roundup® (Glyphosate 41 % EC, SD Fine Chemicals Mumbai, India); this is not representative for the EU renewal.	The RMS agrees with the applicant's justification.
1308	Toxicology and metabolism	Dar M. A. et al.	2019	Effect of Repeated Oral Administration of Roundup((R)) and Ammonium Nitrate on Liver of Wistar Rats.	Proceedings of the Indian National Science Academy Part B Biological Sciences (2019), Vol. 89, No. 2, pp. 505-510	Formulation tested (Roundup, 41% EC) which is not the representative formulation used in the renewal.	The RMS agrees with the applicant's justification.
1309	Toxicology and metabolism	Dardiotis E. et al.	2019	Pesticide exposure and cognitive function: Results from the Hellenic Longitudinal Investigation of Aging and Diet (HELIAD)	Environmental Research (2019), Vol. 177, pp. 108632	This publication is considered not relevant for the risk assessment of glyphosate because exposure to glyphosate is not documented.	The RMS agrees with the applicant's justification.
1310	Toxicology and metabolism	de Adad L. M. M. et al.	2015	Occupational exposure of workers to pesticides: Toxicogenetics and susceptibility gene polymorphisms.	Genetics and Molecular Biology (2015), Vol. 38, No. 3, pp. 308-315	Not specific to glyphosate and therefore not relevant to the renewal.	The RMS agrees with the applicant's justification.
1311	Toxicology and metabolism	de Aguiar L. M. et al.	2016	Glyphosate-based herbicide exposure causes antioxidant defence responses in the fruit fly Drosophila melanogaster.	Comparative biochemistry and physiology. Toxicology & pharmacology (2016), Vol. 185-186, pp. 94-101	Tested formulation (Roundup Original) for cellular mechanisms in houseflies, not directly relevant to human health risk assessment in the EU renewal of glyphosate.	The RMS agrees with the applicant's justification.
1312	Toxicology and metabolism	de Castilhos Ghisi N. et al.	2013	Genotoxic effects of the herbicide Roundup(®) in the fish Corydoras paleatus (Jenyns 1842) after short-term, environmentally low concentration exposure	Environmental monitoring and assessment (2013), Vol. 185, No. 4, pp. 3201-7	Glyphosate based herbicide tested in aquatic species. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.

1313	Toxicology and metabolism	de Melo M. I. A. et al.	2018	Glyphosate-based herbicide induces toxic effects on human adipose-derived mesenchymal stem cells grown in human plasma.	Comparative Clinical Pathology (2018), Vol. 27, No. 4, pp. 989-1000	Glyphosate based herbicide tested in an in vitro system.	The RMS agrees with the applicant's justification.
1314	Toxicology and metabolism	de Liz Oliveira Cavalli V. L. et al.	2013	Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells.	Free radical biology & medicine (2013), Vol. 65, pp. 335-46	Formulation tested in vitro was Roundup Original, 360 g/L, a.e., Brazil. As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1315	Toxicology and metabolism	de Moura F. R. et al.	2017	Effects of glyphosate-based herbicide on pintado da Amazonia: Hematology, histological aspects, metabolic parameters and genotoxic potential.	Environmental toxicology and pharmacology (2017), Vol. 56, pp. 241-248	The effects of high doses of Glyphosate based herbicide to aquatic species was assessed. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1316	Toxicology and metabolism	de Oliveira A. F. B. et al.	2019	Investigation of pesticide exposure by genotoxicological, biochemical, genetic polymorphic and in silico analysis	Ecotoxicology and Environmental Safety (2019), 179, 135-142	This publication is considered not relevant for the risk assessment of glyphosate because it is not specific to glyphosate. Focuses on mixtures of pesticides. It is not possible to establish a causal relationship between the biological endpoints assessed and exposure to glyphosate.	The RMS agrees with the applicant's justification.
1317	Toxicology and metabolism	de Oliveira Joaquim A. et al.	2014	Effects of exposure to glyphosate in male and female mice behavior in pubertal period.	Brazilian Journal of Veterinary Research and Animal Science (2014), Vol. 51, No. 3, pp. 194-203	This publication is considered not relevant for the risk assessment of glyphosate because a formulation (Roundup Transorb) was used instead of glyphosate.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1318	Toxicology and metabolism	de Oliveira Joaquim A. et al.	2012	Behavioral effects of acute glyphosate exposure in male and female Balb/c mice.	Brazilian Journal of Veterinary Research and Animal Science (2012), Vol. 49, No. 5, pp. 367-376	Formulation tested in vivo was Roundup Transorb, 648 g/L of isopropylamine salt, 480 g/L a.e., Brazil. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1319	Toxicology and metabolism	de Ribeiro Sena T. R. et al.	2019	[High frequency hearing among rural workers exposed to pesticides] Audicao em altas frequencias em trabalhadore0s rurais expostos a agrotoxicos	Ciencia & saude coletiva (2019), Vol. 24, No. 10, pp. 3923-3932	No glyphosate specific data, confounded due to multiple pesticide uses therefore cannot be used in the renewal.	The RMS agrees with the applicant's justification.
1320	Toxicology and metabolism	de Souza J. S. et al.	2019	Maternal glyphosate-based herbicide exposure alters antioxidant-related genes in the brain and serum metabolites of male rat offspring.	Neurotoxicology (2019), Vol. 74, pp. 121-131	Formulated product tested was Glyphosate Roundup Transorb; Monsanto of Brazil Ltda, São Paulo, Brazil. As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1321	Toxicology and metabolism	de Souza J. S. et al.	2017	Perinatal exposure to glyphosate-based herbicide alters the thyrotrophic axis and causes thyroid hormone homeostasis imbalance in male rats.	Toxicology (2017), Vol. 377, pp. 25-37	This publication is considered not relevant for the risk assessment of glyphosate because a glyphosate formulation (Roundup Transorb) was used instead of glyphosate.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1322	Toxicology and metabolism	Defarge N. et al.	2018	Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides.	Toxicology reports (2018), Vol. 5, pp. 156-163	This paper is deemed not relevant as a non-representative formulation was tested as opposed to glyphosate.	The RMS agrees with the applicant's justification.

1323	Toxicology and metabolism	Deshmukh U. S. et al.	2013	Effect of acute exposure of glyphosate herbicide, on wistar rats with reference to haematology and biochemical analysis	Bioscan (2013), Vol. 8, No. 2, pp. 381-383	Formulation tested in vivo at excessively high dose of 4000 mg/kg/day for 7 days and is therefore not applicable to the EU renewal.	The RMS agrees with the applicant's justification.
1324	Toxicology and metabolism	Dhananjayan V. et al.	2019	Assessment of genotoxicity and cholinesterase activity among women workers occupationally exposed to pesticides in tea garden	Mutation Research, Genetic Toxicology and Environmental Mutagenesis (2019), Vol. 841, pp. 1-7	General pesticide exposure evaluation, not glyphosate specific therefore not applicable to the EU renewal.	The RMS agrees with the applicant's justification.
1325	Toxicology and metabolism	Dhanarajam Y. et al.	2013	Haemato-biochemical studies on glyphosate induced toxicity in rats.	Journal of Interacademicia (2013), Vol. 17, No. 3, pp. 512-517	Formulation tested in vivo, via oral gavage at high doses of 400 and 800 mg/kg/day for 28 days (Roundup, 41% isopropylamine salt). As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1326	Toxicology and metabolism	Diaz-Criollo S. et al.	2019	Chronic pesticide mixture exposure including paraquat and respiratory outcomes among Colombian farmers.	Industrial health (2019), Vol. 58, No. 1, pp. 15-21	Mixtures paper, focused on paraquat mixtures therefore not relevant to the EU renewal.	The RMS agrees with the applicant's justification.
1327	Toxicology and metabolism	Schrenk D.	2018	What is the meaning of 'A compound is carcinogenic'?.	Toxicology reports (2018), Vol. 5, pp. 504-511	This publication is considered not relevant for the risk assessment of glyphosate as it concerns the classification of carcinogens in general and not glyphosate in particular.	The RMS agrees with the applicant's justification.
1328	Toxicology and metabolism	Diken M. E. et al.	2017	In vitro effects of some pesticides on glutathione-s transferase activity.	Fresenius Environmental Bulletin (2017), Vol. 26, No. 12A, pp. 8023-8029	Formulations tested at excessively high in vitro doses in the mM range and is therefore not applicable to the EU renewal.	The RMS agrees with the applicant's justification.
1329	Toxicology and metabolism	Dimpfel W. et al.	2018	Effect of Zembrin® and four of its alkaloid constituents on electric excitability of the rat hippocampus.	Journal of Ethnopharmacology (2018), Vol. 223, pp. 135-141	AMPA described in the paper is not aminomethylphosphonic acid, rather α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid, therefore not relevant to the renewal.	The RMS agrees with the applicant's justification.
1330	Toxicology and metabolism	Djaldetti R. et al.	2019	The role of exposure to pesticides in the etiology of Parkinson's disease: a 18F-DOPA positron emission tomography study.	Journal of Neural Transmission (2019), Vol. 126, No. 2, pp. 159-166	A general pesticides paper which does not present any new glyphosate specific data. Therefore not relevant to the renewal.	The RMS agrees with the applicant's justification.
1331	Toxicology and metabolism	dos Santos K. C. et al.	2014	Genotoxic and biochemical effects of atrazine and Roundup(®), alone and in combination, on the Asian clam Corbicula fluminea.	Ecotoxicology and environmental safety (2014), Vol. 100, pp. 7-14	A glyphosate based herbicide was tested on an aquatic invertebrate. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1332	Toxicology and metabolism	Douwes J. et al.	2018	Carcinogenicity of glyphosate: why is New Zealand's EPA lost in the weeds?.	The New Zealand medical journal (2018), Vol. 131, No. 1472, pp. 82-89	Opinion article with no new data relevant to the renewal of glyphosate.	The RMS agrees with the applicant's justification.
1333	Toxicology and metabolism	Dumukhalska Y. B. et al.	2018	Protective effect of the cisteile-histidile-tyrosile-histidile- isoleucine against heavy metal and glyfosate induced on content of lipid peroxidation products and reactive oxygen species in different age rats	Medichna ta Klinichna Khimiya (2018), No. 2, pp. 77-83	Administered a glyphosate based herbicide to rats for 30 days at 25% of acute oral LD50, this is not a representative way of exposure.	The RMS agrees with the applicant's justification.
1334	Toxicology and metabolism	Eapen A. et al.	2018	Science, safety, and sanity: hot topics in food toxicology.	Journal of Food Protection (2018), Vol. 81, pp. 24	This paper did not mention glyphosate and is therefore not relevant.	The RMS agrees with the applicant's justification.

1335	Toxicology and metabolism	Elhalwagy M. E. A. et al.	2014	Hepatoxicity induced by glyphosate-based herbicide baron in albino rats.	Journal of Animal and Veterinary Advances (2014), Vol. 13, No. 5, pp. 322-329	Formulation tested in vivo (Baron, 48% glyphosate, Egypt). As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1336	Toxicology and metabolism	Elie-Caille C. et al.	2010	Morphological damages of a glyphosate-treated human keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation.	Cell biology and toxicology (2010), Vol. 26, No. 4, pp. 331-9	This publication is considered not relevant for the risk assessment of glyphosate as the test concentrations used were in the range of 10-70 mM (all >> 1mM) and therefore considered physiologically irrelevant.	The RMS agrees with the applicant's justification.
1337	Toxicology and metabolism	Emmanuel A. G. et al.	2015	Protective potential of betulinic acid against glyphosate-induced toxicity in testis and epididymis of male wistar rats	International Journal of Current Research (2015), Vol. 7, No. 6, pp. 16650-16660	Formulation tested in vivo (decribed as "commercial glyphosate"). As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1338	Toxicology and metabolism	Erhunmwunse N. O. et al.	2014	Histopathological changes in the brain tissue of Africa catfish exposure to glyphosate herbicide.	Journal of Applied Sciences and Environmental Management (2014), Vol. 18, No. 2, pp. 275-280	Formulation tested (commercial formulation of glyphosate (360 g/l-41 w.wt IPA). As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1339	Toxicology and metabolism	Fagan J. et al.	2015	The Seralini affair: degeneration of Science to Re-Science?	Environmental Sciences Europe (2015), Vol. 27, No. 19	Commentary from the Seralini paper retraction therefore not relevant to the renewal.	The RMS agrees with the applicant's justification.
1340	Toxicology and metabolism	Faria M. A.	2015	Glyphosate, neurological diseases - and the scientific method	Surgical neurology international (2015), Vol. 6, pp. 132	A letter providing comments on Samsel and Seneff (ref 2324). Therefore not relevant for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1341	Toxicology and metabolism	Feng P. et al.	2019	A review on gut remediation of selected environmental contaminants: Possible roles of probiotics and gut microbiota.	Nutrients (2019), Vol. 11, No. 1, pp. 22	A literature review on pollutants, probiotics and gut microbes therefore not relevant for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1342	Toxicology and metabolism	Flandroy L. et al.	2018	The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems.	Science of the Total Environment (2018), Vol. 627, pp. 1018-1038	General discussion of microbiota and proposal for research prioritization therefore not relevant for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1343	Toxicology and metabolism	Fluegge K. et al.	2017	Exploring the potential confounder of nitrogen fertilizers in the relationship between pesticide exposures and risk of leukemia: a Poisson regression with two-way fixed-effects analysis	Chinese Journal of Cancer (2017), Vol. 36, No. 1, pp. 58	Letter to editor, focuses on nitrogen fertilizers and is therefore not relevant for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1344	Toxicology and metabolism	Fluegge K. et al.	2017	Exposure to ambient PM10 and nitrogen dioxide and ADHD risk: A reply to Min & Min (2017).	Environment International (2017), Vol. 103, pp. 109-110	No new data, therefore not relevant for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1345	Toxicology and metabolism	Fluegge K. R. et al.	2015	Glyphosate Use Predicts ADHD Hospital Discharges in the Healthcare Cost and Utilization Project Net (HCUPnet): A Two-Way Fixed-Effects Analysis.	PloS one (2015), Vol. 10, No. 8, pp. e0133525	Retracted publication, therefore not relevant for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1346	Toxicology and	Ford B. et al.	2017	Mapping Proteome-wide Targets of Glyphosate in Mice.	Cell chemical biology (2017), Vol. 24, No. 2, pp. 133-140	This publication is considered not relevant because intraperitoneal injection was used	The RMS agrees with the applicant's justification.

	metabolism					which is an inappropriate route of administration for the occupational and food risk assessment of glyphosate.	
1347	Toxicology and metabolism	Freddo N. et al.	2019	Isoflavone quantitation in soymilk: Genistein content and its biological effect.	CyTA-Journal of Food (2019), Vol. 17, No. 1, pp. 20-24	It mainly concerns the development of a bioanalytical method for the analysis of genistein and glyphosate in soya milk. The biological end-point selected (anxiety) and the test system used (elevated plus maze test) are not acceptable for regulatory use.	The RMS agrees with the applicant's justification.
1348	Toxicology and metabolism	Frescura V. D. et al.	2013	Post-treatment with plant extracts used in Brazilian folk medicine caused a partial reversal of the antiproliferative effect of glyphosate in the Allium cepa test	Biocell (2013), Vol. 37, No. 2, pp. 23-8	Glyphosate used as an un-validated positive control in assay and is therefore not relevant for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1349	Toxicology and metabolism	Fu H. et al.	2019	Toxicity of glyphosate in feed for weanling piglets and the mechanism of glyphosate detoxification by the liver nuclear receptor CAR/PXR pathway.	Journal of hazardous materials (2019), Vol. 387, pp. 121707	Glyphosate based herbicide dosed to weanling piglets. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1350	Toxicology and metabolism	Fuso A. et al.	2019	CpG and non-CpG methylation in the diet-epigenetics-neurodegeneration connection.	Current Nutrition Reports (2019), Vol. 8, No. 2, pp. 74-82	A review paper that mentions glyphosate once without any data. Therefore is not relevant for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1351	Toxicology and metabolism	Gallegos C. E. et al.	2016	Exposure to a glyphosate-based herbicide during pregnancy and lactation induces neurobehavioral alterations in rat offspring.	Neurotoxicology (2016), Vol. 53, pp. 20-28	Formulation tested in vivo via drinking water (Glifloglex, 48% glyphosate, Gleba S.R.L., Argentina). As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1352	Toxicology and metabolism	Gallegos C. E. et al.	2018	Perinatal Glyphosate-Based Herbicide Exposure in Rats Alters Brain Antioxidant Status, Glutamate and Acetylcholine Metabolism and Affects Recognition Memory.	Neurotoxicity research (2018), Vol. 34, No. 3, pp. 363-374	Formulation tested (in Argentina, Glifloglex® from Gleba S.R.L., 48 g isopropylamine salt per 100 cm3; 35.6% w/v a.e.). As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1353	Toxicology and metabolism	Gasnier C. et al.	2011	Defined plant extracts can protect human cells against combined xenobiotic effects.	Journal of occupational medicine and toxicology (2011), Vol. 6, No. 1, pp. 3	Protective mechanism of plant extracts upon various chemical exposure (RoundUp residues, Bisphenol A, Atrazine). As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1354	Toxicology and metabolism	Gasnier C. et al.	2010	Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines	Journal of Occupational Medicine and Toxicology (2010), Vol. 5, pp. 29-29	Protective mechanism of plant extracts upon various chemical exposure (RoundUp residues, Bisphenol A, Atrazine). As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1355	Toxicology and metabolism	Gencer N. et al.	2011	In vitro effects of some pesticides on PON1Q192 and PON1R192 isoenzymes from human serum.	Fresenius Environmental Bulletin (2011), Vol. 20, No. 3, pp. 590-596	Test material identity is entirely missing and the claim presented is dubious: "The pesticides were of commercial origin, and at the highest available purity level (99%)." Therefore it is not relevant to the renewal of glyphosate.	The RMS agrees with the applicant's justification.
1356	Toxicology	Gentile N. et al.	2012	Micronucleus assay as a biomarker	Bulletin of Environmental	This paper does not contain glyphosate specific	The RMS agrees with the

	and metabolism			of genotoxicity in the occupational exposure to agrochemicals in rural workers	Contamination and Toxicology (2012), Vol. 88, No. 6, pp. 816-822	data and is therefore not relevant to the renewal of glpyohsate.	applicant's justification.
1357	Toxicology and metabolism	George J. et al.	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach.	Journal of proteomics (2010), Vol. 73, No. 5, pp. 951-64	The test material was a glyphosate-based formulation and not the reference formulation MON 52276. As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1358	Toxicology and metabolism	George J. et al.	2013	Emptying of Intracellular Calcium Pool and Oxidative Stress Imbalance Are Associated with the Glyphosate-Induced Proliferation in Human Skin Keratinocytes HaCaT Cells.	ISRN dermatology (2013), Vol. 2013, pp. 825180	Formulation tested in vivo via dermal application (Roundup Original, 41% isopropylamine salt, 36% a.e.). Relevance of proteomic measurements not validated and as this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1359	Toxicology and metabolism	Gomez A. L. et al.	2019	Male mammary gland development and methylation status of estrogen receptor alpha in Wistar rats are modified by the developmental exposure to a glyphosate-based herbicide.	Molecular and cellular endocrinology (2019), Vol. 481, pp. 14-25	Formulation tested (Magnum Super II, Grupo Agros SA; 66.2% K salt, 54% a e.). As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1360	Toxicology and metabolism	Gomez-Arroyo S. et al.	2013	Assessing the genotoxic risk for Mexican children who are in residential proximity to agricultural areas with intense aerial pesticide applications	Revista Internacional de Contaminacion Ambiental (2013), Vol. 29, No. 3, pp. 217-225	This study does not present any glyphosate specific information and is therefore not relevant to the renewal.	The RMS agrees with the applicant's justification.
1361	Toxicology and metabolism	Goussard P. et al.	2019	Corrosive injury of the trachea in children.	Clinical Case Reports (2019), Vol. 7, No. 10, pp. 1999-2003	One of the cases cited in the article swallowed an unknown amount of glyphosate formulation. No other mention of glyphosate. Focus of article on corrosion of trachea, not glyphosate and is therefore not relevant to the renewal	The RMS agrees with the applicant's justification.
1362	Toxicology and metabolism	Gress S. et al.	2016	Dig1 protects against locomotor and biochemical dysfunctions provoked by Roundup.	BMC complementary and alternative medicine (2016), Vol. 16, pp. 234	Glyphosate based herbicide administered to rats in drinking water. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1363	Toxicology and metabolism	Gress S. et al.	2015	Cardiotoxic Electrophysiological Effects of the Herbicide Roundup(®) in Rat and Rabbit Ventricular Myocardium In Vitro.	Cardiovascular toxicology (2015), Vol. 15, No. 4, pp. 324-35	Roundup Ultra formulation tested in vitro. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1364	Toxicology and metabolism	Guerrero Schimpf M. et al.	2018	Glyphosate-based herbicide enhances the uterine sensitivity to estradiol in rats.	The Journal of endocrinology (2018), Vol. 239, No. 2, pp 197-213	Non representative formulation tested instead of glyphosate. As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1365	Toxicology and metabolism	Guerrero Schimpf M. et al.	2017	Neonatal exposure to a glyphosate based herbicide alters the development of the rat uterus.	Toxicology (2017), Vol. 376, pp. 2-14	Formulation tested in vivo via subcutaneous injection (Roundup FULL II, 66.2% potassium salt). As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.

1366	Toxicology and metabolism	Guha N. et al.	2013	Characterization of residential pesticide use and chemical formulations through self-report and household inventory: The northern California childhood leukemia study.	Environmental Health Perspectives (2013), Vol. 121, No. 2, pp. 276-282	No data relevant to glyphosate human health effects and exposure to glyphosate therefore not relevant to the risk assessments.	The RMS agrees with the applicant's justification.
1367	Toxicology and metabolism	Guilherme S. et al.	2012	DNA damage in fish (Anguilla anguilla) exposed to a glyphosate-based herbicide -- elucidation of organ-specificity and the role of oxidative stress.	Mutation research (2012), Vol. 743, No. 1-2, pp. 1-9	Glyphosate based herbicide tested in eels, surfactants present in the formulation are known to damage gills. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1368	Toxicology and metabolism	Guilherme S. et al.	2014	Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure?.	Aquatic toxicology (2014), Vol. 155, pp. 213-21	Glyphosate based herbicide tested in aquatic species. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1369	Toxicology and metabolism	Gunatilake S. et al.	2019	Glyphosate's Synergistic Toxicity in Combination with Other Factors as a Cause of Chronic Kidney Disease of Unknown Origin.	International journal of environmental research and public health (2019), Vol. 16, No. 15	This publication is considered not relevant for the risk assessment of glyphosate because it does not present concrete epidemiological data on a possible association between chronic kidney disease and a synergistic effect of glyphosate with other environmental factors such as heavy metals.	The RMS agrees with the applicant's justification.
1370	Toxicology and metabolism	Guyton K. Z. et al.	2015	Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate	Lancet Oncology (2015), Vol. 16, no. 5, pp. 490-491	This review is considered not relevant for the risk assessment of glyphosate because it does not contain a detailed report and discussion of experimental results. It only concerns a brief summary of the IARC evaluation of glyphosate which is not corroborated by regulatory agencies.	The RMS agrees with the applicant's justification.
1371	Toxicology and metabolism	Halwachs S. et al.	2016	Assessment of ABCG2-mediated transport of pesticides across the rabbit placenta barrier using a novel MDCKII in vitro model.	Toxicology and applied pharmacology (2016), Vol. 305, pp. 66-74	No adverse effects, and therefore there is no relevance to the human health risk assessment.	The RMS agrees with the applicant's justification.
1372	Toxicology and metabolism	Hamdaoui L. et al.	2016	Nephrotoxicity of Kalach 360 SL: biochemical and histopathological findings.	Toxicology mechanisms and methods (2016), Vol. 26, No. 9, pp. 685-691	Formulation tested (Kalach 360 SL) in vivo. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1373	Toxicology and metabolism	Hamdaoui L. et al.	2019	Sub-chronic exposure to Kalach 360 SL-induced damage in rats' liver and hematological system.	Environmental science and pollution research international (2019), Vol. 26, No. 36, pp. 36634-36646	Glyphosate based herbicide dosed to rats. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1374	Toxicology and metabolism	Hamdaoui L. et al.	2018	Subchronic exposure to kalach 360 SL-induced endocrine disruption and ovary damage in female rats.	Archives of physiology and biochemistry (2018), Vol. 124, No. 1, pp. 27-34	Formulation tested (KL, Arysta Life Science, Fouchana Tunisia; isopropylamine salt 41.5%; surfactant, 15.5%). As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1375	Toxicology and metabolism	Han J. et al.	2016	Determination of glyphosate and its metabolite in emergency room in Korea.	Forensic science international (2016), Vol. 265, pp. 41-6	Analytical method development in human blood therefore not relevant to the glyphosate risk assessment.	The RMS agrees with the applicant's justification.

1376	Toxicology and metabolism	Hao Y. et al.	2019	Roundup confers cytotoxicity through DNA damage and Mitochondria-Associated apoptosis induction	Environmental Pollution (2019), Vol. 252, No. Part_A, pp. 917-923	This publication is considered not relevant for the risk assessment of glyphosate because a glyphosate formulation was tested in vitro instead of glyphosate.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1377	Toxicology and metabolism	Hao Y. et al.	2019	Evaluation of the cytotoxic effects of glyphosate herbicides in human liver, lung, and nerve	Journal of Environmental Science and Health, Part B Pesticides, Food Contaminants, and Agricultural Wastes (2019), Vol. 54, No. 9, pp. 737-744	This publication is considered not relevant for the risk assessment of glyphosate because glyphosate concentrations were tested in vitro that are physiologically not feasible in in vivo experimental models (> 1 mM).	The RMS agrees with the applicant's justification.
1378	Toxicology and metabolism	Haskovic E. et al.	2016	Effects of Glyphosate on Enzyme Activity and Serum Glucose in Rats Rattus norvegicus	Acta veterinaria (2016), Vol. 66, No. 2, pp. 214-221	Only liver enzymes measured after 15 days dermal application of formulated product (Total 480 SL, Croatia), which is not a representative formulation fo the renewal.	The RMS agrees with the applicant's justification.
1379	Toxicology and metabolism	Hendges C. et al.	2019	Human intoxication by agrochemicals in the region of South Brazil between 1999 and 2014.	Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes (2019), Vol. 54, No. 4, pp. 219-225	This publication is considered not relevant for the risk assessment of glyphosate because it does not address specifically glyphosate exposure but pesticide poisoning in general.	The RMS agrees with the applicant's justification.
1380	Toxicology and metabolism	Heritier L. et al.	2017	Oxidative stress induced by glyphosate-based herbicide on freshwater turtles.	Environmental toxicology and chemistry (2017), Vol. 36, No. 12, pp. 3343-3350	Glyphosate based herbicide tested on turtles. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1381	Toxicology and metabolism	Hernandez-Plata I. et al.	2015	The herbicide glyphosate causes behavioral changes and alterations in dopaminergic markers in male Sprague-Dawley rat.	Neurotoxicology (2015), Vol. 46, pp. 79-91	This publication is considered not relevant because of the use of intraperitoneal injection which is an inappropriate route of exposure for the occupational and food risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1382	Toxicology and metabolism	Herrera-Valdes R. et al.	2019	Epidemic of chronic kidney disease of nontraditional etiology in El Salvador: Integrated health sector action and south-south cooperation.	MEDICC Review (2019), Vol. 21, No. 3, pp. 46-52	No data specific to glyphosate. Evaluated handling of agrochemicals as a risk factor, rather than individual pesticides.	The RMS agrees with the applicant's justification.
1383	Toxicology and metabolism	Heu C. et al.	2012	Glyphosate-induced stiffening of HaCaT keratinocytes, a Peak Force Tapping study on living cells.	Journal of structural biology (2012), Vol. 178, No. 1, pp. 1-7	This publication is considered not relevant for the risk assessment of glyphosate because glyphosate concentrations have been used in vitro that cannot be attained in in vivo experimental models (> 1 mM).	The RMS agrees with the applicant's justification.
1384	Toxicology and metabolism	Heu C. et al.	2012	A step further toward glyphosate-induced epidermal cell death: involvement of mitochondrial and oxidative mechanisms.	Environmental toxicology and pharmacology (2012), Vol. 34, No. 2, pp. 144-153	This publication is considered not relevant for the risk assessment of glyphosate because the cytotoxicity of glyphosate to epidermal cells was tested in the mM range whereas contact of epidermal cells to glyphosate formulations is always combined with surfactants which produce cytotoxicity in the sub-mM range.	The RMS agrees with the applicant's justification.
1385	Toxicology and metabolism	Hofmann J. N. et al.	2015	The Biomarkers of Exposure and Effect in Agriculture (BEEA) Study: Rationale, Design, Methods, and Participant Characteristics	Journal of toxicology and environmental health. Part A (2015), Vol. 78, No. 21-22, pp. 1338-47	No endpoints for glyphosate, only relative use rates therefore cannot be used in the risk assessments.	The RMS agrees with the applicant's justification.
1386	Toxicology	Hong S. et al.	2012	Cellular Toxicity of Surfactants	JOURNAL OF KOREAN	This study does not present any glyphosate	The RMS agrees with the

	and metabolism			Used as Herbicide Additives	MEDICAL SCIENCE (2012), Vol. 27, No. 1, pp. 3-9	specific information and cannot therefore be used in risk assessments.	applicant's justification.
1387	Toxicology and metabolism	Hong Y. et al.	2017	Effects of glyphosate on immune responses and haemocyte DNA damage of Chinese mitten crab, Eriocheir sinensis.	Fish & shellfish immunology (2017), Vol. 71, pp. 19-27	This paper discusses the effects of high doses of a glyphosate based herbicideto crabs. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1388	Toxicology and metabolism	Hsu C. et al.	2013	Can mortality from agricultural pesticide poisoning be predicted in the emergency department? Findings from a hospital-based study in eastern Taiwan	Tzu Chi Medical Journal (2013), Vol. 25, no. 1, pp. 32-38	This paper provides a retrospective analysis of poisoning incidents in Taiwan and is therefore not relevant to the renewal of glyphosate.	The RMS agrees with the applicant's justification.
1389	Toxicology and metabolism	Hulin M. et al.	2014	Assessment of infant exposure to food chemicals: the French Total Diet Study design	Food Additives & Contaminants, Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment (2014), Vol. 31, No. 7, pp. 1226-1239	This paper describes the assessment process of infant exposure to food chemicals. No glyphosate data was presented in the report, and is therefore not relevant to the renewal process.	The RMS agrees with the applicant's justification.
1390	Toxicology and metabolism	Hussain R. et al.	2019	Exposure to Sub-Acute Concentrations of Glyphosate Induce Clinico-Hematological, Serum Biochemical and Genotoxic Damage in Adult Cockerels	PAKISTAN VETERINARY JOURNAL (2019), Vol. 39, No. 2, pp. 181-186	The glyphosate based herbicide used in the paper is not an EU representative formulation and is therefore not relevant to the renewal. Furthermore, the product was administered via gavage to avian species.	The RMS agrees with the applicant's justification.
1391	Toxicology and metabolism	Hutter H. et al.	2018	Cytotoxic and Genotoxic Effects of Pesticide Exposure in Male Coffee Farmworkers of the Jarabacoa Region, Dominican Republic	INTERNATIONAL JOURNAL OF ENVIRONMENTAL RESEARCH AND PUBLIC HEALTH (2018), Vol. 15, No. 8	This study did not include any analyses specific for glyphosate, so it is not relevant.	The RMS agrees with the applicant's justification.
1392	Toxicology and metabolism	IARC	2017	Some organophosphate insecticides and herbicides.	IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (2017), Vol. 112, VII + pp. 452	This paper provides a secondary source of infomation and is therefore not relevant.	The RMS agrees with the applicant's justification.
1393	Toxicology and metabolism	Ibrahim A. M. et al.	2019	Toxicological impact of butralin, glyphosate-isopropylammonium and pendimethalin herbicides on physiological parameters of Biomphalaria alexandrina snails	Molluscan research (2019), Vol. 39, No. 3, pp. 224-233	This paper describes an ecotoxicology study of snails exposed to a glyphosate based herbicide. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1394	Toxicology and metabolism	Ikpeme E. V. et al.	2012	Efficacy of ascorbic acid in reducing glyphosate-induced toxicity in rats.	British Biotechnology Journal (2012), Vol. 2, No. 3, pp. 157-168	The formulation tested in vivo is not described. It is not sure what was tested and therefore the effect cannot be attributed to glyphosate.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1395	Toxicology and metabolism	Ilyushina N. A. et al.	2019	Applicability of the Ames test and micronucleus test in vivo for the evaluation of the equivalence of pesticide technical grade active ingredients compared to original active substances	Gigiena i Sanitariya (2019), No. 2, pp. 219-224	Technical grade glyphosate was used as positive control in an assay within this paper. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1396	Toxicology	Ingaramo P. I. et	2019	Acute uterine effects and long-term	Food and chemical toxicology	A glyphosate based herbicide was dosed to rats	RMS requested a study summary in

	and metabolism	al.		reproductive alterations in postnatally exposed female rats to a mixture of commercial formulations of endosulfan and glyphosate.	(2019), Vol. 134, pp. 110832	in this report. As this is not the representative formulation, the article is not relevant to the renewal.	order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1397	Toxicology and metabolism	Ingaramo P. I. et al.	2017	Neonatal exposure to a glyphosate-based herbicide alters uterine decidualization in rats.	Reproductive toxicology (2017), Vol. 73, pp. 87-95	Formulation tested in vivo via sub-cutaneous injection (undisclosed brand, 66.2% potassium salt; 54% glyphosate acid). As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1398	Toxicology and metabolism	Ingaramo P. I. et al.	2016	Effects of neonatal exposure to a glyphosate-based herbicide on female rat reproduction.	Reproduction (2016), Vol. 152, No. 5, pp. 403-15	A glyphosate based herbicide formulation was tested in vivo (66.2%, potassium salt). As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1399	Toxicology and metabolism	Intranuovo G. et al.	2018	Assessment of DNA damages in lymphocytes of agricultural workers exposed to pesticides by comet assay in a cross-sectional study	Biomarkers (2018), Vol. 23, No. 5, pp. 462-473	General pesticide exposure evaluation, not glyphosate specific. Therefore this article is not relevant to the glyphosate renewal process.	The RMS agrees with the applicant's justification.
1400	Toxicology and metabolism	Iummato M. M. et al.	2017	Effect of glyphosate acid on biochemical markers of periphyton exposed in outdoor mesocosms in the presence and absence of the mussel Limnoperna fortunei.	Environmental toxicology and chemistry (2017), Vol. 36, No. 7, pp. 1775-1784	The end-points described in this study are not relevant to human health risk assessments in the renewal.	The RMS agrees with the applicant's justification.
1401	Toxicology and metabolism	Jayasumana C.	2019	Chronic Interstitial Nephritis in Agricultural Communities (CINAC) in Sri Lanka	SEMINARS IN NEPHROLOGY (2019), Vol. 39, No. 3, pp. 278-283	There is no evaluation of glyphosate exposure with any disease outcome presented in this paper. Therefore it is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1402	Toxicology and metabolism	Jayasumana C. et al.	2015	Drinking well water and occupational exposure to Herbicides is associated with chronic kidney disease, in Padavi-Sripura, Sri Lanka.	Environmental health (2015), Vol. 14, pp. 6	This study was performed in Sri Lanka and is therefore not relevant to the EU.	The RMS agrees with the applicant's justification.
1403	Toxicology and metabolism	Jayasumana C. et al.	2015	Phosphate fertilizer is a main source of arsenic in areas affected with chronic kidney disease of unknown etiology in Sri Lanka.	SpringerPlus (2015), Vol. 4, pp. 90	No data on glyphosate is presented, and is therefore not relevant to the renewal dossier.	The RMS agrees with the applicant's justification.
1404	Toxicology and metabolism	Ji H. et al.	2018	Differential microRNA expression in the prefrontal cortex of mouse offspring induced by glyphosate exposure during pregnancy and lactation.	Experimental and therapeutic medicine (2018), Vol. 15, No. 3, pp. 2457-2467	In this paper a glyphosate based formulation was tested, (purchased in China) and containing 48% IPA salt, and 35.6% a.e. Furthermore a glyphosate-based formulation (marketed in China) was used instead of glyphosate in an in vivo assay in mice with the end-points measured not suitable for risk assessment (differential microRNA expression in the prefrontal cortex).	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1405	Toxicology and metabolism	Jiang X. et al.	2018	A commercial Roundup formulation induced male germ cell apoptosis by promoting the expression of XAF1	Toxicology Letters (2018), Vol. 296, pp. 163-172	In this study, a Roundup formulation was administered via gavage to adult male mice. As this is not the representative formulation, the	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1

				in adult mice		article is not relevant to the renewal.	of Volume 3 CA B.6.6 for RMS comments.
1406	Toxicology and metabolism	Kamata R. et al.	2018	Agonistic effects of diverse xenobiotics on the constitutive androstane receptor as detected in a recombinant yeast-cell assay	Toxicology In Vitro (2018), Vol. 46, pp. 335-349	This paper presents yeast cell assay validation. Glyphosate was not active in the test system and therefore this is not relevant for the renewal.	The RMS agrees with the applicant's justification.
1407	Toxicology and metabolism	Kamel F. et al.	2012	Pesticide exposure and amyotrophic lateral sclerosis	NeuroToxicology (2012), Vol. 33, No. 3, pp. 457-462	This study does not present correlations of glyphosate use and effect and is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1408	Toxicology and metabolism	Karthikraj R. et al.	2019	Widespread occurrence of glyphosate in urine from pet dogs and cats in New York State, USA.	The Science of the total environment (2019), Vol. 659, pp. 790-795	These data do not meet any data requirement under Regulation (EC) 1107/2009 and do not fit within any standard risk assessment under that regulation. The results indicate that exposure to glyphosate may be common in this limited population of companion animals, but at levels which do not raise toxicological concern.	The RMS agrees with the applicant's justification.
1409	Toxicology and metabolism	Kawada T.	2018	Glyphosate toxicity and carcinogenicity.	EXCLI journal (2018), Vol. 17, pp. 800-801	Letter to editor citing other publications in this review, with no data discussed. Therefore not relevant to the renewal.	The RMS agrees with the applicant's justification.
1410	Toxicology and metabolism	Khayat C. B. et al.	2013	Assessment of DNA damage in Brazilian workers occupationally exposed to pesticides: a study from Central Brazil.	Environmental Science and Pollution Research International (2013), Vol. 20, No. 10, pp. 7334-7340	No specific analyses was performed for glyphosate in this paper. Furthermore, uncertain sampling from an undefined population and adequate statistical analysis was carried out. No description of a case control study was provided, and the analysis did not evaluate a causal parameter for case control studies (e.g., an odds ratio) or address potential biases in the analysis. Therefore this study is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1411	Toxicology and metabolism	Kim S. et al.	2019	Pesticides as a risk factor for metabolic syndrome: Population-based longitudinal study in Korea	Molecular & Cellular Toxicology (2019), Vol. 15, No. 4, pp. 431-441	Epidemiology study on pesticide use in general. No information on specific pesticides used in the study was collected and is therefore not applicable to the renewal of glyphosate.	The RMS agrees with the applicant's justification.
1412	Toxicology and metabolism	Kongtip P. et al.	2018	A cross-sectional investigation of cardiovascular and metabolic biomarkers among conventional and organic farmers in Thailand	International Journal of Environmental Research and Public Health (2018), Vol. 15, No. 11, pp. 2590	This paper presents an evalution of the effects of pesticide use in general on metabolic biomarkers. The results are not correlated to glyphosate use and cannot be used in glyphosate risk assessments.	The RMS agrees with the applicant's justification.
1413	Toxicology and metabolism	Koutros S. et al.	2013	Genetic susceptibility loci, pesticide exposure and prostate cancer risk	PLoS One (2013), Vol. 8, No. 4, pp. e58195	This paper does not mention glyphosate and is not relevant.	The RMS agrees with the applicant's justification.
1414	Toxicology and metabolism	Kubsad D. et al.	2019	Assessment of Glyphosate Induced Epigenetic Transgenerational Inheritance of Pathologies and Sperm Epimutations: Generational Toxicology	Scientific Reports (2019), Vol. 9, No. 1, pp. 1-17	This publication is considered not relevant because the intraperitoneal route of administration is not appropriate for the risk assessment of glyphosate.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1415	Toxicology and	Kumar V. et al.	2018	Interactions of Acephate, Glyphosate, Monocrotophos and	Indian Journal of Pharmaceutical Sciences	Study of binding to bovine serum albumin by several pesticides. No significant effect of	The RMS agrees with the applicant's justification.

	metabolism			Phorate with Bovine Serum Albumin.	(2018), Vol. 80, No. 6, pp. 1151-1154	glyphosate in this test system and is therefore not relevant for the risk assessment.	
1416	Toxicology and metabolism	Kurenbach B. et al.	2017	Herbicide ingredients change Salmonella enterica sv. Typhimurium and Escherichia coli antibiotic responses.	Microbiology (2017), Vol. 163, pp. 1791-1801	This study describes the addition of high doses of herbicide ingredients to an in vitro system. The reason for the exclusion of in vitro testing of formulations to assess health effects as a result of systemic exposure is the presence of surfactants which produce cell toxicity based on the destabilization of the cell membrane and the mitochondrial membrane thus masking the specific toxicity of glyphosate.	The RMS agrees with the applicant's justification.
1417	Toxicology and metabolism	Kwiatkowska M. et al.	2014	The effect of metabolites and impurities of glyphosate on human erythrocytes (in vitro).	Pesticide biochemistry and physiology (2014), Vol. 109, pp. 34-43	This publication is considered not relevant for the risk assessment of glyphosate because the in vitro concentrations used are in the mM range and the impurities were tested at the same concentrations as glyphosate which will never occur in practice.	The RMS agrees with the applicant's justification.
1418	Toxicology and metabolism	Kwiatkowska M. et al.	2016	The Impact of Glyphosate, Its Metabolites and Impurities on Viability, ATP Level and Morphological changes in Human Peripheral Blood Mononuclear Cells.	PloS one (2016), Vol. 11, No. 6, pp. e0156946	This publication is considered not relevant for the risk assessment of glyphosate because the in vitro concentrations used are in the mM range and the impurities were tested at the same concentrations as glyphosate which will never occur in practice.	The RMS agrees with the applicant's justification.
1419	Toxicology and metabolism	Lajmanovich R. C. et al.	2015	Harmful Effects of the Dermal Intake of Commercial Formulations Containing Chlorpyrifos, 2,4-D, and Glyphosate on the Common Toad Rhinella arenarum (Anura: Bufonidae).	Water Air and Soil Pollution (2015), Vol. 226, No. 12, pp. Article No.: 427	This study describes toad dermal exposure to a glyphosate based herbicide. Dermal uptake via moist toad skin was assessed and the end-points identified are not relevant to the human health risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1420	Toxicology and metabolism	Landrigan P. J.	2018	Pesticides and Human Reproduction.	JAMA Internal Medicine (2018), Vol. 178, No. 1, pp. 26-27	No data provided in this paper as it is a commentary article. Cannot be used in a glyphosate risk assessment.	The RMS agrees with the applicant's justification.
1421	Toxicology and metabolism	Larsen K. E. et al.	2016	The herbicide glyphosate is a weak inhibitor of acetylcholinesterase in rats.	Environmental toxicology and pharmacology (2016), Vol. 45, pp. 41-4	This publication is considered not relevant for the risk assessment of glyphosate because the concentrations used for in vitro testing were all in the mM range and not representative of in use conditions.	The RMS agrees with the applicant's justification.
1422	Toxicology and metabolism	Larsson M. O. et al.	2018	Corrigendum to "Refined assessment and perspectives on the cumulative risk resulting from the dietary exposure to pesticide residues in the Danish population"[Food and Chemical Toxicology 111 (2018) 207-267] [Erratum to document cited in CA169:146371]	Food and Chemical Toxicology (2018), Vol. 113, pp. 345-346	Corrigendum to paper correcting calculations not pertaining to glyphosate. Therefore not relevant.	The RMS agrees with the applicant's justification.
1423	Toxicology and metabolism	Lee H. M. et al.	2012	A case of activated charcoal aspiration treated by early and repeated bronchoalveolar lavage.	Tuberculosis and Respiratory Diseases (2012), Vol. 72, No. 2, pp. 177-181	Effects attributed to activated charcoal aspiration, not glyphosate. Therefore the paper is not relevant to the renewal.	The RMS agrees with the applicant's justification.

1424	Toxicology and metabolism	Lee J-W. et al.	2015	Common Pesticides Used in Suicide Attempts Following the 2012 Paraquat Ban in Korea.	Journal of Korean medical science (2015), Vol. 30, No. 10, pp. 1517-21	Reports numbers of suicide attempts in South Korea, common pesticide use, not specifically referring to glyphosate.	The RMS agrees with the applicant's justification.
1425	Toxicology and metabolism	Lermen J. et al.	2018	Pesticide exposure and health conditions among orange growers in Southern Brazil	Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes (2018), Vol. 53, No. 4, pp. 215-221	This publication is not relevant for the risk assessment of glyphosate because the biological monitoring data were only used to address pesticide exposure in general and not glyphosate in particular.	The RMS agrees with the applicant's justification.
1426	Toxicology and metabolism	Leveroni F. A. et al.	2017	Genotoxic response of blood, gill and liver cells of Piaractus mesopotamicus after an acute exposure to a glyphosate-based herbicide	Caryologia (2017), Vol. 70, No. 1, pp. 21-28	Formulation tested in aquatic species (Roundup Full II; 66.2% glyphosate potassium salt; CAS no. 70901-12-1). As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1427	Toxicology and metabolism	Lewis M. M. et al.	2017	Lateralized basal ganglia vulnerability to pesticide exposure in asymptomatic agricultural workers	Toxicological Sciences (2017), Vol. 159, No. 1, pp. 170-178	The results presented are not correlated with exposure to glyphosate. Therefore this article is not relevant to the renewal of glyphosate.	The RMS agrees with the applicant's justification.
1428	Toxicology and metabolism	Leyva-Soto L. A. et al.	2018	GLYPHOSATE AND AMINOMETHYLPHOSPHONIC ACID IN POPULATION OF AGRICULTURAL FIELDS: HEALTH RISK ASSESSMENT OVERVIEW.	Applied Ecology and Environmental Research (2018), Vol. 16, No. 4, pp. 5127-5140	This paper is misrepresented as an epidemiologic cohort study. It is an informal community health risk survey with uncertain exposure assessment and uncertain health outcomes. The study population is poorly characterized. Uncertain temporal relationship between purported glyphosate drinking water exposure and disease outcome. The analysis was not appropriate for a cohort (or case control) study and is not relevant for glyphosate renewal.	The RMS agrees with the applicant's justification.
1429	Toxicology and metabolism	Li M-H. et al.	2016	Multi-tissue metabolic responses of goldfish (Carassius auratus) exposed to glyphosate-based herbicide.	Toxicology Research (2016), Vol. 5, No. 4, pp. 1039-1052	This paper presents results of a glyphosate based herbicide tested on goldfish. The end-points defined are not relevant to the human health risk assessment for glyphosate renewal.	The RMS agrees with the applicant's justification.
1430	Toxicology and metabolism	Li Q. et al.	2013	Glyphosate and AMPA inhibit cancer cell growth through inhibiting intracellular glycine synthesis.	Drug design, development and therapy (2013), Vol. 7, pp. 635-43	This paper described the theraputic applicactions of glyphosate and AMPA at very high in vitro doses to cancer cells. This is deemed not relevant to the glyphosate renewal.	The RMS agrees with the applicant's justification.
1431	Toxicology and metabolism	Li Z.	2018	The use of a disability-adjusted life-year (DALY) metric to measure human health damage resulting from pesticide maximum legal exposures.	Science of the Total Environment (2018), Vol. 639, pp. 438-456	This publication is considered not relevant because it concerns the development of a uniform metric (the disability-adjusted life-year; DALY) in risk characterisation to express the human health impact of pesticide exposure and not experimental data that can be used for the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
1432	Toxicology and metabolism	Litvinko N. M. et al.	2015	The effect of N-(phosphonomethyl)-glycine on phospholytic reaction catalyzed by phospholipase A2	Vestsi Natsyyanal'nai Akademii Navuk Belarusi, Seryya Khimichnykh Navuk (2015), Vol. 3, pp. 91-100	Unrealistic in vitro concentrations of ≥ 100 mg/mL were tested in the study. Therefore not relevant to the renewal of glyphosate.	The RMS agrees with the applicant's justification.
1433	Toxicology and	Loomba R. S.	2016	Prevalence of isomerism from a European registry: Live births, fetal	Congenital Anomalies (2016), Vol. 56, No. 6, pp. 256-257	This paper does not mention glyphosate or AMPA and is not relevant.	The RMS agrees with the applicant's justification.

	metabolism			deaths, and terminations of pregnancy.			
1434	Toxicology and metabolism	Lopez Gonzalez E. C. et al.	2013	Induction of micronuclei in broad snouted caiman (Caiman latirostris) hatchlings exposed in vivo to Roundup® (glyphosate) concentrations used in agriculture	Pesticide biochemistry and physiology (2013), Vol. 105, No. 2, pp. 131-134	The formulation tested in reptiles (Roundup, undefined, uncharacterized) is not the representative formulation, and therefore the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1435	Toxicology and metabolism	Lorenz V. et al.	2019	Epigenetic disruption of estrogen receptor alpha is induced by a glyphosate-based herbicide in the preimplantation uterus of rats.	Molecular and cellular endocrinology (2019), Vol. 480, pp. 133-141	Formulation tested (MAGNUM SUPER II) marketed in Argentina by Grupo Agros S.R.L. and comprises 66.2% potassium salt and 54% w/v a.e. As this is not the representative formulation, the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1436	Toxicology and metabolism	Loro V. L. et al.	2015	Glyphosate-based herbicide affects biochemical parameters in Rhamdia quelen (Quoy & Gaimard, 1824 and) Leporinus obtusidens (Valenciennes, 1837).	Neotropical Ichthyology (2015), Vol. 13, No. 1, pp. 229-235	This study describes the application of high aquatic doses of glyphosate based herbicide with observed effects attributable to the surfactant present in the formulation. As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1437	Toxicology and metabolism	Luaces J. P. et al.	2017	Genotoxic effects of Roundup Full II® on lymphocytes of Chaetophractus villosus (Xenarthra, Mammalia): In vitro studies.	PloS one (2017), Vol. 12, No. 8, pp. e0182911	Formulation tested in vivo (Roundup Full II, containing 66.2% glyphosate, Argentina). As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1438	Toxicology and metabolism	Luo L. et al.	2017	In vitro cytotoxicity assessment of roundup (glyphosate) in L-02 hepatocytes.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2017), Vol. 52, No. 6, pp. 410-417	Formulation tested in vitro (Roundup, containing 41% isopropylamine salt; Belgium). The effects observed are due to high dosing of the surfactant in vitro, and as this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1439	Toxicology and metabolism	Mahakhode R. H. et al.	2013	Mitotic abnormalities induced by glyphosate in Psoralea corylifolia L..	International Journal of Current Pharmaceutical Research (2013), Vol. 5, No. 1, pp. 46-48	Tested a plant species with a herbicide for adverse end-points; the identified end-points are not relevant to human health and the renewal.	The RMS agrees with the applicant's justification.
1440	Toxicology and metabolism	Malagoli C. et al.	2016	Passive exposure to agricultural pesticides and risk of childhood leukemia in an Italian community.	International journal of hygiene and environmental health (2016), Vol. 219, No. 8, pp. 742-748	This study did not perform any specific analyses for glyphosate. Furthermore, there was a very small case control study with a speculative exposure variable. This is not relevant for the renewal of glyphosate.	The RMS agrees with the applicant's justification.
1441	Toxicology and metabolism	Mao Y. et al.	2015	Effect of glyphosate on serum biochemical indices of exposed workers	Zhongguo Gongye Yixue Zazhi (2015), Vol. 28, No. 5, pp. 362-364	The worker protections and manufacturing processes in China do not reflect Western occupational exposure scenarios. Therefore this is not relevant to glyphosate renewal.	The RMS agrees with the applicant's justification.
1442	Toxicology and metabolism	Marcoccia D. et al.	2017	Food components and contaminants as (anti)androgenic molecules.	Genes and Nutrition (2017), Vol. 12, No. 1 pp. 6	This paper discusses some glyphosate literature, but does not provide new data. Therefore it cannot be used in the glyphosate risk assessments.	The RMS agrees with the applicant's justification.
1443	Toxicology and metabolism	Marques A. et al.	2014	Progression of DNA damage induced by a glyphosate-based herbicide in fish (Anguilla anguilla)	Comparative biochemistry and physiology. Toxicology & pharmacology (2014), Vol. 166,	This study outlines the test of a glyphosate based herbicide to aquatic species. As this is not the representative formulation, the article is not	The RMS agrees with the applicant's justification.

				upon exposure and post-exposure periods--insights into the mechanisms of genotoxicity and DNA repair.	pp. 126-33	relevant to the renewal.	
1444	Toxicology and metabolism	Martinez M. et al.	2019	Use of human neuroblastoma SH-SY5Y cells to evaluate glyphosate-induced effects on oxidative stress, neuronal development and cell death signaling pathways.	Environment international (2019), Vol. 135, pp. 105414	This publication is considered not relevant for the risk assessment of glyphosate and AMPA as the concentrations used for the measurement of oxidative stress and apoptosis were beyond the physiologically acceptable range of 1 mM (5 and 10 mM).	The RMS agrees with the applicant's justification.
1445	Toxicology and metabolism	Martini C. N. et al.	2012	A commercial formulation of glyphosate inhibits proliferation and differentiation to adipocytes and induces apoptosis in 3T3-L1 fibroblasts.	Toxicology in vitro (2012), Vol. 26, No. 6, pp. 1007-13	The formulation tested in vitro (commercial glyphosate formulation; 48% w/v, isopropylamine salt,from Atanor, Argentina) is not the representative formulation, and thus the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1446	Toxicology and metabolism	Martini C. N. et al.	2016	Glyphosate Inhibits PPAR Gamma Induction and Differentiation of Preadipocytes and is able to Induce Oxidative Stress.	Journal of biochemical and molecular toxicology (2016), Vol. 30, No. 8, pp. 404-13	Formulation tested in vitro at a single high dose in the mM range (Glifosato Atanor, containing 48% isopropylamine salt, 35.6% glyphosate, Argentina). As this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1447	Toxicology and metabolism	Martini C. N. et al.	2016	Glyphosate-based herbicides with different adjuvants are more potent inhibitors of 3T3-L1 fibroblast proliferation and differentiation to adipocytes than glyphosate alone.	Comparative Clinical Pathology (2016), Vol. 25, No. 3, pp. 607-613	In this paper, three glyphosate-based herbicides with different adjuvants were tested in vitro. Glyphosate only effects were noted only at excessively high doses > 20mM, this is physiologically not possible to attain in standard regulatory in vivo testing	The RMS agrees with the applicant's justification.
1448	Toxicology and metabolism	Marx-Stoelting P. et al.	2014	Assessment of three approaches for regulatory decision making on pesticides with endocrine disrupting properties	Regulatory Toxicology and Pharmacology (2014), Vol. 70, No. 3, pp. 590-604	No glyphosate specific information was presented in this paper and therefore this article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1449	Toxicology and metabolism	Mesnage R. et al.	2013	Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide.	Journal of applied toxicology (2013), Vol. 33, No. 7, pp. 695-9	Not only was the glyphosate based herbicide formulation tested together with other substances (Roundup GT Plus containing 450 g/L glyphosate), this is not the representative formulation, the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1450	Toxicology and metabolism	Mesnage R. et al.	2015	Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure.	Environmental health (2015), Vol. 14, article No. 70	Formulation tested (Grand Travaux Plus (450 g/L, Belgium) for non-validated endpoints therefore cannot be used in an EU Annex I renewal.	The RMS agrees with the applicant's justification.
1451	Toxicology and metabolism	Mesnage R. et al.	2018	Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide (vol 7, 39328, 2017).	Scientific Reports (2018), Vol. 8, pp. Article No.: 12572	The Roundup formulation tested in rats is not the representative formulation, and the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1452	Toxicology and	Meyer-Monath M. et al.	2014	Development of a multi-residue method in a fetal matrix: analysis of	Analytical and Bioanalytical Chemistry (2014), Vol. 406,	This is primarily an analytical method paper for determination of multiple analytes (including	RMS requested a study summary in order to further justify the

	metabolism			meconium	No. 30, pp. 7785-7797	glyphosate) in meconium. Actual meconium samples were analyzed. Minimal details of results provided, and no detections of glyphosate reported, therefore the report is not relevant.	categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1453	Toxicology and metabolism	Moreno N. C. et al.	2014	Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish Prochilodus lineatus.	Environmental toxicology and pharmacology (2014), Vol. 37, No. 1, pp. 448-54	Formulation tested (Roundup Transorb® containing480 g glyphosate /L, Monsanto Brazil Ltd). As this is not the representative formulation, and the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1454	Toxicology and metabolism	Morley W. A. et al.	2014	Diminished brain resilience syndrome: A modern day neurological pathology of increased susceptibility to mild brain trauma, concussion, and downstream neurodegeneration.	Surgical neurology international (2014), Vol. 5, pp. 97	Many hypotheses are discussed in this studywith no data presented that could be used in a renewal dossier.	The RMS agrees with the applicant's justification.
1455	Toxicology and metabolism	Moshammer H. et al.	2019	Validity of reported indicators of pesticide exposure and relevance for cytotoxic and genotoxic effects on buccal cells.	Mutagenesis (2019), Vol. 34, No. 2, pp. 147-152	This publication is considered not relevant for the risk assessment of glyphosate as the association between pesticide use in general and genotoxicity and cytotoxicity markers in buccal cells was studied only, and not specifically glyphosate.	The RMS agrees with the applicant's justification.
1456	Toxicology and metabolism	Murussi C. et al.	2014	Changes in oxidative markers, endogenous antioxidants and activity of the enzyme acetylcholinesterase in farmers exposed to agricultural pesticides - a pilot study	Ciencia Rural (2014), Vol. 44, No. 7, pp. 1186-1193	This pilot study evaluated the use of general pesticides only with a comparison between treated and non-treated. Glyphosate alone was not evaluated and as a result this study cannot be used in the renewal dossier.	The RMS agrees with the applicant's justification.
1457	Toxicology and metabolism	Mwabulambo S. G. et al.	2018	Health symptoms associated with pesticides exposure among flower and onion pesticide applicators in Arusha region.	Annals of Global Health (2018), Vol. 84, No. 3, pp. 369-379	This document describes the use of PPE during general occupational pesticide use and is not relevant to the glyphosate renewal dossier.	The RMS agrees with the applicant's justification.
1458	Toxicology and metabolism	Nagy K. et al.	2020	Systematic review of comparative studies assessing the toxicity of pesticide active ingredients and their product formulations.	Environmental research (2020), Vol. 181, pp. 108926	This is a review paper with no new data presented. Therefore is not relevant to the glyphosate risk assessments.	The RMS agrees with the applicant's justification.
1459	Toxicology and metabolism	Nardi J. et al.	2017	Prepubertal subchronic exposure to soy milk and glyphosate leads to endocrine disruption.	Food and chemical toxicology (2017), Vol. 100, pp. 247-252	A paper describing a glyphosate formulation co-dosed with phytoestrogen. As this is a mixture the effects cannot be determined for glyphosate alone and thus the paper is not relevant for the renewal dossier.	The RMS agrees with the applicant's justification.
1460	Toxicology and metabolism	Naz S. et al.	2019	Effect of glyphosate on hematological and biochemical parameters of Rabbit (Oryctolagus cuniculus)	Pure and Applied Biology (2019), Vol. 8, No. 1, pp. 78-92	Rabbits gavaged with a glyphosate based herbicide(Glyphosate comprised 48% of the formulation with another 48% glyphosate IPA (isopropylammonium) salt) sourced in Pakistan. As this is not the representative formulation, and the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1461	Toxicology and	Negga R. et al.	2012	Exposure to glyphosate- and/or Mn/Zn-ethylene-bis-	Neurotoxicity research (2012), Vol. 21, No. 3, pp. 281-90	This study describes invertebrate tests performed using a glyphosate based herbicide.	The RMS agrees with the applicant's justification.

	metabolism			dithiocarbamate-containing pesticides leads to degeneration of γ-aminobutyric acid and dopamine neurons in Caenorhabditis elegans.		As this is not the representative formulation, and the article is not relevant to the renewal.	
1462	Toxicology and metabolism	Nippanon P. et al.	2019	Chemical pesticide use and quality of life of rubber farmers in the Northeast of Thailand.	Kathmandu University Medical Journal (2019), Vol. 17, No. 65	A paper evaluating the handling of agrochemicals as a risk factor, rather than individual pesticides. Similarly, only the percentage of farmers using glyphosate was reported.	The RMS agrees with the applicant's justification.
1463	Toxicology and metabolism	Nishiyori Y. et al.	2014	Unilateral hippocampal infarction associated with an attempted suicide: a case report.	Journal of medical case reports (2014), Vol. 8, pp. 219	Glyphosate does not cross the blood brain barrier and does not cause neurotoxicity. Nor would it be expected that glyphosate ingestion unilaterally targets the dorsal part of the left hippocampus. This presentation is much more consistent with a small vessel embolic event and the patient should have been evaluated for risk factors for stroke such as atrial fibrillation or carotid atherosclerosis. Not relevant for the risk assessment.	The RMS agrees with the applicant's justification.
1464	Toxicology and metabolism	Nobels I. et al.	2011	Toxicity Ranking and Toxic Mode of Action Evaluation of Commonly Used Agricultural Adjuvants on the Basis of Bacterial Gene Expression Profiles	PLOS ONE (2011), Vol. 6, No. 11, pp. E24139	A study on commonly used adjuvants and solvents in pesticide formulations, however no glyphosate or Roundup specific data is mentioned therefore the study is not relevant for the renewal dossier.	The RMS agrees with the applicant's justification.
1465	Toxicology and metabolism	Norskov N. P. et al.	2019	Robust and highly sensitive micro liquid chromatography-tandem mass spectrometry method for analyses of polar pesticides (glyphosate, aminomethylphosfonic acid, N-acetyl glyphosate and N-acetyl aminomethylphosfonic acid) in multiple biological matrices.	Journal of chromatography. A (2019), Vol. 1605, pp. 360343	This paper concerns development of a glyphosate assay and is likely a precursor to a gut microbe study. No animal data and no information relevant for the risk assessment were presented.	The RMS agrees with the applicant's justification.
1466	Toxicology and metabolism	Nur G. et al.	2018	Histopathological and biochemical responses to the oxidative stress induced by glyphosate-based herbicides in the rainbow trout (Oncorhynchus mykiss)	Journal of Cellular Neuroscience and Oxidative Stress (2018), Vol. 10, No. 1, pp. 656-665	A glyphosate based herbicidewas tested in aquatic species, without positive control to verify validity of the assay. Gill damage is directly attributable to the surfactant present in the formulation, with oxidative stress a consequence of cell damage.	The RMS agrees with the applicant's justification.
1467	Toxicology and metabolism	Nwani C. D. et al.	2014	Induction of micronuclei and nuclear lesions in Channa punctatus following exposure to carbosulfan, glyphosate and atrazine.	Drug and chemical toxicology (2014), Vol. 37, No. 4, pp. 370-7	A glyphosate based herbicide (Roundup SL; India; 41% soluble liquid) was tested in aquatic species. This study discusses cellular and molecular level end-points that are not relevant to an EU level ecotoxicology risk assessment.	The RMS agrees with the applicant's justification.
1468	Toxicology and metabolism	Owrang I. et al.	2013	Antioxidant effect of ginger on the pituitary-gonadal axis hormones recovered from the devastating effects of the herbicide Glyphosate in female rats	International Journal of Biology, Pharmacy and Allied Sciences (2013), Vol. 2, No. 8, pp. 1606-1616	Dosing via i.p. injection daily for three weeks is not relevant. The glyphosate source is not described at all. It is not clear whether this study dosed a glyphosate based herbicide, a technical acid or salt.	The RMS agrees with the applicant's justification.

1469	Toxicology and metabolism	Pandey A. et al.	2019	Inflammatory Effects of Subacute Exposure of Roundup in Rat Liver and Adipose Tissue.	Dose-response (2019), Vol. 17, No. 2, pp. 1	The formulation tested in this study (Herbicide Roundup, 41% w/w glyphosate, Monsanto India Ltd, Mumbai, India) is not the representative formulation and is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1470	Toxicology and metabolism	Parajuli K. R. et al.	2015	Aminomethylphosphonic acid and methoxyacetic acid induce apoptosis in prostate cancer cells.	International journal of molecular sciences (2015), Vol. 16, No. 5, pp. 11750-65	In this study the therapeutic use of AMPA was evaluated rather than glyphosate. Therefore it is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1471	Toxicology and metabolism	Parajuli K. R. et al.	2016	Aminomethylphosphonic acid inhibits growth and metastasis of human prostate cancer in an orthotopic xenograft mouse model.	Oncotarget (2016), Vol. 7, No. 9, pp. 10616-26	In this study the therapeutic use of AMPA was evaluated rather than glyphosate. Therefore it is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1472	Toxicology and metabolism	Paumgartten F. J. R.	2019	Comment on 'Perinatal exposure to a glyphosate-based herbicide impairs female reproductive outcomes and induces second-generation adverse effects in Wistar rats', Arch Toxicol 92:2629-2643 : On the impairment of female reproductive performance by developm	Archives of toxicology (2019), Vol. 93, No. 3, pp. 831-832	Letter refers to a previous paper published by Milesi et al (Perinatal exposure to a glyphosate-based herbicide impairs.; Arch Toxicol 2018, 92(8):2629–2643.): Milesi et al is not in Marian's LRR2 list.	The RMS agrees with the applicant's justification.
1473	Toxicology and metabolism	Perego M. C. et al.	2017	Influence of a Roundup formulation on glyphosate effects on steroidogenesis and proliferation of bovine granulosa cells in vitro.	Chemosphere (2017), Vol. 188, pp. 274-279	This study examines the in vitro formulation effects only, rather than glyphosate alone.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1474	Toxicology and metabolism	Perez-Torres I. et al.	2017	Beneficial Effects of the Amino Acid Glycine.	Mini reviews in medicinal chemistry (2017), Vol. 17, No. 1, pp. 15-32	This paper does not contain any data pertaining to glyphosate and is therefore not relevant.	The RMS agrees with the applicant's justification.
1475	Toxicology and metabolism	Peters C. E. et al.	2018	Priority Setting for Occupational Cancer Prevention.	Safety and Health at Work (2018), Vol. 9, No. 2, pp. 133-139	This paper does not contain any data pertaining to glyphosate and is therefore not relevant	The RMS agrees with the applicant's justification.
1476	Toxicology and metabolism	Portier C. J. et al.	2016	Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA).	Journal of epidemiology and community health (2016), Vol. 70, No. 8, pp. 741-5	This publication is considered not relevant because it is not based on experimental data.	The RMS agrees with the applicant's justification.
1477	Toxicology and metabolism	Pouokam G. B. et al.	2017	A Pilot Study in Cameroon to Understand Safe Uses of Pesticides in Agriculture, Risk Factors for Farmers' Exposure and Management of Accidental Cases.	Toxics (2017), Vol. 5, No. 4	This paper does not contain any data pertaining to glyphosate and is therefore not relevant.	The RMS agrees with the applicant's justification.
1478	Toxicology and metabolism	Qiu S. et al.	2020	Toxic effects of glyphosate on intestinal morphology, antioxidant capacity and barrier function in weaned piglets.	Ecotoxicology and environmental safety (2020), Vol. 187, pp. 109846	This study investigates glyphosate based herbicides dosed to piglets. As this is not the representative formulation, and the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1479	Toxicology and metabolism	Ramsden J. J.	2017	Assaults on health.	Journal of Biological Physics and Chemistry (2017), Vol. 17, No. 1, pp. 3-7	Commentary on various threats to human health and not directly relevant to the renewal.	The RMS agrees with the applicant's justification.

1480	Toxicology and metabolism	Rappazzo K. M. et al.	2019	Maternal residential exposure to specific agricultural pesticide active ingredients and birth defects in a 2003-2005 North Carolina birth cohort.	Birth defects research (2019), Vol. 111, No. 6, pp. 312-323	Highly speculative exposure assessment limited to pesticides makes it impossible to adequately assess results. Therefore this study is not relevant.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1481	Toxicology and metabolism	Robert J. R. et al.	2013	Council on Environmental Health. Technical Report: Pesticide Exposure in Children (vol 130, pg e1765, 2012).	Pediatrics (2013), Vol. 131, No. 5, pp. 1013-1014	This paper does not contain any data pertaining to glyphosate and is therefore not relevant.	The RMS agrees with the applicant's justification.
1482	Toxicology and metabolism	Rojas Garcia A. E. et al.	2018	Special issue on pesticide contamination and toxicology. Numero especial: Contaminacion y toxicologia por plaguicidas	Revista Internacional de Contaminacion Ambiental (2018), Vol. 34, pp. 7-105	A special issue with seven articles that were either reviews discussing glyphosate, or notrelevant to glyphosate.	The RMS agrees with the applicant's justification.
1483	Toxicology and metabolism	Romano M. A. et al.	2012	Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression.	Archives of toxicology (2012), Vol. 86, No. 4, pp. 663-73	This publication is considered not relevant for the risk assessment of glyphosate as a glyphosate based herbicide (Roundup Transorb) has been tested instead of glyphosate.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1484	Toxicology and metabolism	Romano M. A. et al.	2012	Reply to comment of John M. DeSesso and Amy L. Williams regarding "Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression" by Romano et al. 2012	Archives of Toxicology (2012), Vol. 86, No. 11, pp. 1795-1797	This article reflects the categorization of the original article which was classified as not relevant.	The RMS agrees with the applicant's justification.
1485	Toxicology and metabolism	Romano R. M. et al.	2010	Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology.	Archives of toxicology (2010), Vol. 84, No. 4, pp. 309-17	The test material was a glyphosate-based formulation and not the reference formulation MON 52276 and is therefore not relevant	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1486	Toxicology and metabolism	Roongruangchai J. et al.	2018	The teratogenic effects of glyphosate based herbicide (GBH) on the development of chick embryos.	Siriraj Medical Journal (2018), Vol. 70, No. 5, pp. 419-428	This report studies the injection of glyphosate based fertilisers into fertilized chicken eggs. As this is not the representative formulation, athe article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix to Volume 3 CA B.9 for RMS comments and summary.
1487	Toxicology and metabolism	Salaroli L. et al.	2019	Occupational Exposure to Agrochemicals, Risks and Safety Practices in Family Agriculture in a Municipality of the State of Espirito Santo, Brazil (P04-077-19).	Current developments in nutrition (2019), Vol. 3, No. Suppl 1, pp. 259	General pesticide review of occupational exposures and safety practices and not relevant for the renewal.	The RMS agrees with the applicant's justification.
1488	Toxicology and metabolism	Samsel A. et al.	2013	Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance.	Interdisciplinary toxicology (2013), Vol. 6, No. 4, pp. 159-84	This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
1489	Toxicology and metabolism	Samsel A. et al.	2015	Glyphosate, pathways to modern diseases III: Manganese, neurological diseases, and associated pathologies.	Surgical neurology international (2015), Vol. 6, pp. 45	This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.

1490	Toxicology and metabolism	Samsel A. et al.	2013	Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases	Entropy (2013), Vol. 15, pp. 1416-1463	This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
1491	Toxicology and metabolism	Samsel A. et al.	2015	Glyphosate, pathways to modern diseases IV: cancer and related pathologies.	Journal of Biological Physics and Chemistry (2015), Vol. 15, No. 3, pp. 121-159	This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
1492	Toxicology and metabolism	Samsel A. et al.	2016	Glyphosate pathways to modern diseases V: Amino acid analogue of glycine in diverse proteins.	Journal of Biological Physics and Chemistry (2016), Vol. 16, No. 1, pp. 9-46	This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
1493	Toxicology and metabolism	Samsel A. et al.	2017	Glyphosate pathways to modern diseases VI: Prions, amyloidoses and autoimmune neurological diseases.	Journal of Biological Physics and Chemistry (2017), Vol. 17, No. 1, pp. 8-32	This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
1494	Toxicology and metabolism	Scammell M. K. et al.	2019	Environmental and Occupational Exposures in Kidney Disease.	Seminars in Nephrology (2019), Vol. 39, No. 3, pp. 230-243	This paper does not contain any new data pertaining to glyphosate and is therefore not relevant.	The RMS agrees with the applicant's justification.
1495	Toxicology and metabolism	Schaumburg L. G. et al.	2016	Genotoxicity induced by Roundup® (Glyphosate) in tegu lizard (Salvator merianae) embryos.	Pesticide biochemistry and physiology (2016), Vol. 130, pp. 71-78	A glyphosate based herbicide was tested in lizard eggs. As this was not the representative formulation the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1496	Toxicology and metabolism	Seneff S. et al.	2015	Death as a drug side effect in FAERS: is glyphosate contamination a factor?	Agricultural Sciences (2015), Vol. 6, No. 12, pp. 1472-1501	Within this report, hypotheses are discussed without any empirical data. Therefore it is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1497	Toxicology and metabolism	Seneff S. et al.	2015	Aluminum and glyphosate can synergistically induce pineal gland pathology: connection to gut dysbiosis and neurological disease.	Agricultural Sciences (2015), Vol. 6, No. 1, pp. 42-70	This paper is not relevant as it is not based on experimental work and no epidemiologic methodology was followed. Conclusion (glyphosate and aluminium, operate synergistically to induce dysfunction in the pineal gland leading to the sleep disorder that is characteristic of multiple neurological diseases, including autism, ADHD, depression, Alzheimer's disease, ALS, anxiety disorder and Parkinson's disease) is pure speculation and is not corroborated by current experimental data.	The RMS agrees with the applicant's justification.
1498	Toxicology and metabolism	Seneff S. et al.	2017	Can glyphosate's disruption of the gut microbiome and induction of sulfate deficiency explain the epidemic in gout and associated diseases in the industrialized world?.	Journal of Biological Physics and Chemistry (2017), Vol. 17, No. 2, pp. 53-76	Study is not relevant as it is not based on experimental work and no epidemiologic methodology was followed. Results and proposed mode of actions are speculation without any experimental proof.	The RMS agrees with the applicant's justification.
1499	Toxicology and metabolism	Seneff S. et al.	2013	Is encephalopathy a mechanism to renew sulfate in autism?	Entropy (2013), Vol. 15, pp. 372-406	This paper presents hypotheses without data and is not relevant.	The RMS agrees with the applicant's justification.
1500	Toxicology and	Seralini G. E.	2015	Why glyphosate is not the issue with Roundup A short overview of 30	Journal of Biological Physics and Chemistry (2015), Vol. 15,	A review of glyphosate vs formulations. Secondary source of information, not	The RMS agrees with the applicant's justification.

	metabolism			years of our research.	No. 3, pp. 111-119	experimental data presented.	
1501	Toxicology and metabolism	Seralini G. E. et al.	2014	Conclusiveness of toxicity data and double standards	FOOD AND CHEMICAL TOXICOLOGY (2014), Vol. 69, pp. 357-359	This is a commentary of a Seralini paper retraction and not relevant.	The RMS agrees with the applicant's justification.
1502	Toxicology and metabolism	Seralini G. E. et al.	2014	Conflicts of interests, confidentiality and censorship in health risk assessment: the example of an herbicide and a GMO.	Environmental Sciences Europe (2014), Vol. 26, No. 13, pp. 1	This is a commentary article and not relevant.	The RMS agrees with the applicant's justification.
1503	Toxicology and metabolism	Seralini G. et al.	2012	Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize.	Food and chemical toxicology (2012), Vol. 50, No. 11, pp. 4221-31	This paper is a retraction announcement from the journal publishers and not relevant.	The RMS agrees with the applicant's justification.
1504	Toxicology and metabolism	Seralini G. et al.	2014	Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize.	Environmental sciences Europe (2014), Vol. 26, No. 1, pp. 14	This publication is considered not relevant for risk assessment of glyphosate because a glyphosate formulation was used instead of glyphosate.	The RMS agrees with the applicant's justification.
1505	Toxicology and metabolism	Siddiqui S. et al.	2012	Glyphosate, alachor and maleic hydrazide have genotoxic effect on Trigonella foenum-graecum L	Bulletin of environmental contamination and toxicology (2012), Vol. 88, No. 5, pp. 659-65	Not valid to evaluate genotoxicity of herbicides on the plant species tested.	The RMS agrees with the applicant's justification.
1506	Toxicology and metabolism	Soudani N. et al.	2019	Glyphosate disrupts redox status and up-regulates metallothionein I and II genes expression in the liver of adult rats. Alleviation by quercetin.	General physiology and biophysics (2019), Vol. 38, No. 2, pp. 123-134	This publication is considered not relevant for the risk assessment of glyphosate because the route of administration used was not appropriate (intraperitoneal injection).	The RMS agrees with the applicant's justification.
1507	Toxicology and metabolism	Stur E. et al.	2019	Glyphosate-based herbicides at low doses affect canonical pathways in estrogen positive and negative breast cancer cell lines.	PloS one (2019), Vol. 14, No. 7, pp. e0219610	This publication is considered not relevant for the risk assessment of glyphosate as the concentration of AMPA used (10 mM) is beyond the physiologically acceptable range (> 1 mM). Evaluation of a glyphosate-based herbicide in in vitro systems is not relevant to the risk assessment of glyphosate due to the effects of surfactants on cells.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1508	Toxicology and metabolism	Swanson N. L. et al.	2014	Genetically engineered crops, glyphosate and the deterioration of health in the United States of America.	Journal of Organic Systems (2014), Vol. 9, No. 2, pp. 6-37	This publication is considered not relevant for the risk assessment of glyphosate as no epidemiological approach was followed to establish an association between exposure to glyphosate and disease outcome.	The RMS agrees with the applicant's justification.
1509	Toxicology and metabolism	Szabo R. et al.	2017	Studies on joint toxic effects of a glyphosate herbicide (Fozat 480) and a heavy metal (cadmium) on chicken embryos.	AGROFOR International Journal (2017), Vol. 2, No. 3, pp. 37-43	Glyphosate based herbicide applied to fertilized chicken eggs, is not the representative formulation and not relevant to human health risk assessment.	The RMS agrees with the applicant's justification.
1510	Toxicology and metabolism	Szemeredy G. et al.	2016	TOXICITY TEST OF INDIVIDUAL AND COMBINED TOXIC EFFECTS OF HERBICIDE GLIALKA STAR AND LEAD-ACETATE ON CHICKEN EMBRYOS. Original Title: GLIALKA STAR GYOMIRTO SZER ES AZ OLOM-ACETAT	Novenyvdelem (2016), Vol. 52, No. 10, pp. 483-487	Formulation tested via injection to chicken embryos. This is not a typical route of exposure. Tested formulation was not the representative formulation for the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix to Volume 3 CA B.9 for RMS comments and summary.

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1511	Toxicology and metabolism	Szepanowski F. et al.	2018	Glyphosate-based herbicide, but not pure glyphosate, affects peripheral nervous system myelination.	European Journal of Neurology (2018), Vol. 25, Supp. 2, pp. 567.	Effects observed in this study were noted with a glyphosate based herbicide only in vitro. In addition, as this was not the representative formulation the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1512	Toxicology and metabolism	Teleken J. L. et al.	2019	Glyphosate-based herbicide exposure during pregnancy and lactation malprograms the male reproductive morphofunction in F1 offspring.	Journal of developmental origins of health and disease (2019), Vol. 11, No. 2, pp 146-153	This study described a glyphosate based herbicidedosed to mice. As this was not the representative formulation the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1513	Toxicology and metabolism	Tincher C. et al.	2017	The Glyphosate-Based Herbicide Roundup Does not Elevate Genome-Wide Mutagenesis of Escherichia coli.	G3 (Bethesda, Md.) (2017), Vol. 7, No. 10, pp. 3331-3335	This publication is considered not relevant for the risk assessment of glyphosate as a glyphosate based formulation was used instead of glyphosate for in vitro testing.	The RMS agrees with the applicant's justification.
1514	Toxicology and metabolism	Tizhe E. et al.	2018	Pancreatic function and histoarchitecture in Wistar rats following chronic exposure to Bushfire®: the mitigating role of zinc.	The Journal of international medical research (2018), Vol. 46, No. 8, pp. 3296-3305	The formulation tested (BushfireVR, containing 441 g/L potassium salt; 360 g a.e./L) is not the representative formulation the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1515	Toxicology and metabolism	Tizhe E. V. et al.	2014	Serum biochemical assessment of hepatic and renal functions of rats during oral exposure to glyphosate with zinc.	Comparative clinical pathology (2014), Vol. 23, pp. 1043-1050	This publication is considered not relevant for the risk assessment of glyphosate as a combination of zinc chloride and a glyphosate formulation (Bushfire) has been used instead of glyphosate.	The RMS agrees with the applicant's justification.
1516	Toxicology and metabolism	Tizhe E. V. et al.	2019	Effect of zinc on erythrocyte osmotic fragility and hemogram following chronic exposure to glyphosate-based herbicide in Wistar rats	Comparative Clinical Pathology (2019), Vol. 28, pp. 1275-1279	Formulation tested (BUSHFIRE, Ningbo Agro-star Industrial Co., Ltd., Zhejiang, China; 441 g/L potassium salt, 360 g/L a.e.). As this was not the representative formulation the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1517	Toxicology and metabolism	Tsatsakis A. M. et al.	2019	Hormetic Neurobehavioral effects of low dose toxic chemical mixtures in real-life risk simulation (RLRS) in rats	Food and Chemical Toxicology (2019), 125, 141-149	The test material was a mixture of thirteen different chemicals and cannot be interpreted for glyphosate alone.	The RMS agrees with the applicant's justification.
1518	Toxicology and metabolism	Turkmen R. et al.	2019	Prenatal and neonatal exposure to glyphosate-based herbicide reduces the primordial to primary follicle transition in the newborn rat ovary: a preliminary study	Kocatepe Veterinary Journal (2019), Vol. 12, No. 2, pp. 168-177	Rats gavaged with a glyphosate based herbicide [Knockdown 48 SL; Safa Agriculture Inc., Turkey] As this was not the representative formulation the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1519	Toxicology and metabolism	Turkmen R. et al.	2019	Protective effects of resveratrol on biomarkers of oxidative stress, biochemical and histopathological changes induced by sub-chronic oral glyphosate-based herbicide in rats.	Toxicology research (2019), Vol. 8, No. 2, pp. 238-245	This paper describes high oral gavage dosing of a glyphosate based herbicide. As this is not the representative formulation the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1520	Toxicology and	Turkmen R. et al.	2019	Antioxidant and cytoprotective effects of N-acetylcysteine against	Environmental science and pollution research international	The formulation tested in this article (Knockdown 48SL, Safa Agriculture Corp.,	The RMS agrees with the applicant's justification.

	metabolism			subchronic oral glyphosate-based herbicide-induced oxidative stress in rats.	(2019), Vol. 26, No. 11, pp. 11427-11437	Turkey; containing 480 g/L isopropylamine salt) is not the representative formulation and is not relevant to the renewal.	
1521	Toxicology and metabolism	Upadhyay J. et al.	2019	Biomarker responses (serum biochemistry) in pregnant female wistar rats and histopathology of their neonates exposed prenatally to pesticides	Brazilian Journal of Pharmaceutical Sciences (2019), Vol. 55, pp. e18194	In this report a glyphosate based herbicide was tested (Topper 77; Crystal Crop Protection Pvt. Ltd. India) at one dose to six rats and compared with controls. As this was not the representative formulation the article is not relevant to the renewal.	The RMS agrees with the applicant's justification.
1522	Toxicology and metabolism	Vandenberg L. N. et al.	2017	Is it time to reassess current safety standards for glyphosate-based herbicides?.	Journal of epidemiology and community health (2017), Vol. 71, No. 6, pp. 613-618	This publication is considered not relevant because it is not based on experimental work.	The RMS agrees with the applicant's justification.
1523	Toxicology and metabolism	Varayoud J. et al.	2017	Effects of a glyphosate-based herbicide on the uterus of adult ovariectomized rats.	Environmental toxicology (2017), Vol. 32, No. 4, pp. 1191-1201	This publication is considered not relevant for the risk assessment of glyphosate as a non-representative glyphosate formulation was tested instead of glyphosate.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1524	Toxicology and metabolism	Vinceti M. et al.	2017	Pesticide exposure assessed through agricultural crop proximity and risk of amyotrophic lateral sclerosis.	Environmental Health (2017), Vol. 16, No. 1. pp. 91	This article does not demonstrate correlations with glyphosate use and effect and is therefore not relevant.	
1525	Toxicology and metabolism	Von Ehrenstein O. S. et al.	2019	Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: Population based case-control study	BMJ (Online) (2019), Vol. 364, pp. l962	This publication is not relevant for the risk assessment of glyphosate with relation to ED because the pathology investigated is not ED related (autism spectrum disorder in children).	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1526	Toxicology and metabolism	Wallace Hayes A.	2014	Editor in Chief of Food and Chemical Toxicology answers questions on retraction	Food and chemical toxicology (2014), Vol. 65, pp. 394-5	Letter from editor on Seralini retraction (2012) -> #2472, 3617, 5654 (all not relevant)	The RMS agrees with the applicant's justification.
1527	Toxicology and metabolism	Wang F. et al.	2018	Advance on clinical study of glyphosate toxicity.	Journal of Environmental & Occupational Medicine (2018), Vol. 35, No. 2, pp. 175-179	A review and summary of selected literature.	The RMS agrees with the applicant's justification.
1528	Toxicology and metabolism	Wilhelm C. M. et al.	2015	Assessment of DNA damage in floriculturists in southern Brazil	Environmental Science and Pollution Research (2015), Vol. 22, No. 11, pp. 8182-8189	No glyphosate specific conclusions, confounded due to multiple pesticide uses.	The RMS agrees with the applicant's justification.
1529	Toxicology and metabolism	Wilke R. A. et al.	2019	Chronic Kidney Disease in Agricultural Communities	AMERICAN JOURNAL OF MEDICINE (2019), Vol. 132, No. 10, pp. E727-E732	Discussion of different factors that could be used to predict prevalence of chronic kidney disease in the US.	The RMS agrees with the applicant's justification.
1530	Toxicology and metabolism	Witherspoon N. O.	2019	Protecting children from known pesticides exposures: our collective duty to provide primary prevention.	Pediatric Research (2019), Vol. 85, No. 2, pp. 118-119	Study does not include data on glyphosate and is therefore not relevant.	The RMS agrees with the applicant's justification.
1531	Toxicology and metabolism	Wongta A. et al.	2018	The Pesticide Exposure of People Living in Agricultural Community, Northern Thailand.	Journal of toxicology (2018), Vol. 2018, pp. 4168034	Farming practices in Thailand are not applicable to European farmer exposure scenarios.	The RMS agrees with the applicant's justification.
1532	Toxicology and metabolism	Wozniak E. et al.	2018	The mechanism of DNA damage induced by Roundup 360 PLUS, glyphosate and AMPA in human peripheral blood mononuclear cells - genotoxic risk assessement.	Food and chemical toxicology (2018), Vol. 120, pp. 510-522	In vitro effects only noted at excessively high doses greater than 100-250 uM. Therefore this article is not relevant to the risk assessment.	The RMS agrees with the applicant's justification.

1533	Toxicology and metabolism	Wumbei A. et al.	2019	Pesticides use and exposure among yam farmers in the Nanumba traditional area of Ghana.	Environmental monitoring and assessment (2019), Vol. 191, No. 5, pp. 307	Article is not relevant to agricultural practices and glyphosate uses in Europe.	The RMS agrees with the applicant's justification.
1534	Toxicology and metabolism	Youness E. R. et al.	2016	The protective effect of orange juice on glyphosate toxicity in adult male mice.	Journal of Chemical and Pharmaceutical Research (2016), Vol. 8, No. 3, pp. 13-28	This study uses excessively high gavage doses to rats and is not relevant to renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1535	Toxicology and metabolism	Yu H. et al.	2013	The antagonistic effects of tea polyphenols on damage of mouse Sertoli cells induced by glyphosate.	Acta Nutrimenta Sinica (2013), Vol. 35, No. 3, pp. 283-287	In vitro study testing of what appears to be a formulated product, described as, glyphosate (41% Isopropylamine Hydrochloride, Monsanto glyphosate (41% Isopropylamine Hydrochloride, Monsanto), dosed at 10-160 ug/mL (high glyphosate levels of 24-390 uM plus surfactant), well above any potential physiological concentrations in sertoli cells.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1536	Toxicology and metabolism	Yu N. et al.	2018	Circular RNA expression profiles in hippocampus from mice with perinatal glyphosate exposure.	Biochemical and biophysical research communications (2018), Vol. 501, No. 4, pp. 838-845	This publication is considered not relevant for risk assessment of glyphosate with relation to ED because a no ED related endpoint was investigated (circular RNA expression profiles in the hippocampus).	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1537	Toxicology and metabolism	Zanardi M. V. et al.	2019	Glyphosate-based herbicide induces hyperplastic ducts in the mammary gland of aging Wistar rats.	Molecular and cellular endocrinology (2019), Vol. 501, pp. 110658	This study examines the effects of glyphosate based herbicide dosed to rats. As this is not the representative formulation the article is not relevant to the renewal.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1538	Toxicology and metabolism	Zhang H.-C. et al.	2018	Molecular cloning, characterization, expression and enzyme activity of catalase from planarian Dugesia japonica in response to environmental pollutants.	Ecotoxicology and environmental safety (2018), Vol. 165, pp. 88-95	Novel microbial test system of questionable relevance to human health risk assessment.	The RMS agrees with the applicant's justification.
1539	Toxicology and metabolism	Zhao W. et al.	2013	Effects of glyphosate on apoptosis and expressions of androgen-binding protein and vimentin mRNA in mouse Sertoli cells	Journal of Southern Medical University (2013), Vol. 33, No. 11, pp. 1709-13	Not relevant. In vitro testing of a glyphosate based herbicide.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1540	Toxicology and metabolism	Zhao W-H. et al.	2016	THE PROTECTIVE EFFECTS OF TEA POLYSACCHARIDES ON INJURY AND APOPTOSIS OF MOUSE SERTOLY CELLS INDUCED BY GLYPHOSATE.	Current Topics in Nutraceutical Research (2016), Vol. 14, No. 1, pp. 81-90	Not relevant. In vitro testing of a glyphosate based herbicide.	RMS requested a study summary in order to further justify the categorization. Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments and summary.
1541	Toxicology and metabolism	Zhu J. et al.	2018	An Effective Machine Learning Approach for Identifying the Glyphosate Poisoning Status in Rats Using Blood Routine Test	IEEE ACCESS (2018), Vol. 6, pp. 15653-15662	Test substance souce not identified, not clear whether glyphosate or formulation administered to animals. Data used in development of machine learning.	The RMS agrees with the applicant's justification.
1542	Toxicology and metabolism	Zoccali C.	2017	Causal mechanism and component causes in Mesoamerican-Sri Lankan nephropathy: the moderator's view	NEPHROLOGY DIALYSIS TRANSPLANTATION (2017), Vol. 32, No. 4, pp. 607-610	No glyphosate specific information provided.	The RMS agrees with the applicant's justification.

Table B.6.10-4b: Overview of articles excluded after detailed assessment (i.e. not relevant) – *top up literature search 2020*

Submission Number	Technical section	Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria)	RMS comments
58	Toxicology and metabolism	Alarcon R. *et al.*	2020	Neonatal exposure to a glyphosate-based herbicide alters the uterine differentiation of prepubertal ewe lambs.	Environmental pollution, (2020) Vol. 265, No. Pt B, Art. No. 114874	Roundup FULL II® (Argos SRL, Santa Fe, Argentina), a liquid water-soluble formulation containing 54 g of glyphosate in 100 mL of commercial formulation is not the EU representative formulation for the EU glyphosate renewal and therefore not relevant to the EU glyphosate renewal. In addition, direct injection into the neck of pregnant ewes with formulated product containing surfactant is not relevant to real life exposure scenarios. Environmental fate, metabolism and pharmaco-kinetics for glyphosate active ingredient versus sufractants are very different. Given the direct systemic exposure, these data are considered irrelevant to livestock and human health risk assessments.	Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments.
59	Toxicology and metabolism	Barbasz A. *et al.*	2020	Toxicity of pesticides toward human immune cells U-937 and HL-60.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes, (2020) pp. 1-7; Doi: 10.1080/03601234.2020.1777059	Human histiocytic lymphoma cell line (U-937) and human promyelocytic cell line (HL-60) were exposed to glyphosate at a concentrations from 1.3 mM to 21.3 mM. Although the data are accurate, the doses selected are not physiologically relevant to human health risk assessment. Effects are noted well in excess of physiologically relevant doses and therefore, are not applicable to the risk assessment.	The RMS agrees with the applicant's justification.
60	Toxicology and metabolism	Calvo-Trujillo M. *et al.*	2019	Exposure to pesticides as a risk factor for Parkinson's disease: A case-control study in San Juan Nepomuceno Town (Bolivar).	Revista de Toxicologia, (2019) Vol. 36, No. 2, pp. 142-147	This publication describes an association between pesticide exposure and Parkinson's disease. The only context in which glyphosate is mentioned is to say that it is a widely used herbicide. There is no claim that glyphosate exposure is associated with PD and therefore this article is not relevant for the glyphosate EU renewal.	The RMS agrees with the applicant's justification.
61	Toxicology and metabolism	Coppola L. *et al.*	2020	Integrated approach to evaluate the association between exposure to pesticides and idiopathic premature thelarche in girls: The PEACH project.	International Journal of Molecular Sciences, (2020) Vol. 21, No. 9, Art. No. 3282	No results are provided in the article, only a section with expected results is included. The article does not provide any new information that can be used in the risk assessment.	The RMS agrees with the applicant's justification.

Submission Number	Technical section	Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria)	RMS comments
62	Toxicology and metabolism	de Castilhos Ghisi N. *et al.*	2020	Glyphosate and its toxicology: A scientometric review.	The Science of the total environment, (2020) Vol. 733, Art. No 139359	This scientometric review does not focus on toxicological endpoints following a glyphosate exposure of any kind. No relevant information or conclusion on the toxicity of glyphosate for the risk assessment can be drawn.	The RMS agrees with the applicant's justification.
63	Toxicology and metabolism	Devault D. A. *et al.*	2020	Wastewater-based epidemiology approach to assess population exposure to pesticides: a review of a pesticide pharmacokinetic dataset	Environmental science and pollution research international (2020) Vol. 27, No. 5, pp. 4695-4702	This publication is a literature review and does not contain any toxicological endpoints following the exposure to glyphosate. It rather aims to identify from literature if it is possible to use waste-water based epidemiology to assess human exposure to different pesticides. The article cannot contribute to the risk assessment of glyphosate.	The RMS agrees with the applicant's justification.
64	Toxicology and metabolism	Djaber N. *et al.*	2020	Roundup-induced biochemical and histopathological changes in the liver and kidney of rats: the ameliorative effects of Linum usitatissimum oil.	Acta Biochimica Polonica, (2020) Vol. 67, No. 1, pp. 53-64	Roundup TURBO (450 g/L) is not the EU representative formulation and therefore not relevant to the EU glyphosate renewal. The Roundup dosing rationale in the study appeared to be to intentionally elicit toxic effects and endorse the administration of Linum usitatissimum oil (LuO) to counter toxicty to liver/kidney tissue (perhaps preventive?). With this approach the dosing regimine is a major flaw in the study design as only one dose level was selected and it is unclear what effects may have been seen, if any, at more relevant exposure levels.	The RMS agrees with the applicant's justification.
65	Toxicology and metabolism	Gallegos C. E. *et al.*	2020	Intranasal glyphosate-based herbicide administration alters the redox balance and the cholinergic system in the mouse brain.	Neurotoxicology, (2020) Vol. 77, pp. 205-215	Glifloglex®, marketed in Argentina, is not the EU representative formulation and therefore not relevant to the EU glyphosate renewal.	The RMS agrees with the applicant's justification.
66	Toxicology and metabolism	Gomez A. L. *et al.*	2020	Exposure to a Glyphosate-based Herbicide Alters the Expression of Key Regulators of Mammary Gland Development on Pre-pubertal Male Rats.	Toxicology, (2020) Vol. 439, Art No. 152477	In vivo study on pre and post natal effects of Glyphosate-based herbicide administered to Wistar female rat at 3.5 and 350 mg/kg/day (8-10 females rat/group). Glyphosate-based herbicide (Glyphosate 66.2% - potassium salt, acid equivalent 54%) was tested, which is not the EU representative formulation and thus article is not relevant for the EU glyphosate renewal.	Refer to Appendix 1 of Volume 3 CA B.6.6 for RMS comments.
67	Toxicology and metabolism	Hamdaoui L. *et al.*	2020	Sub-chronic exposure to Kalach 360 SL, Glyphosate-based Herbicide, induced bone rarefaction in female	Toxicology, (2020) Vol. 436, Art. No. 152412	In vivo study on effects of Kalach 360 SL on ED parameters on wistar female rat at 2 different doses. Kalach 360 SL is not the EU representative formulation and thus article is	The RMS agrees with the applicant's justification.

Submission Number	Technical section	Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria)	RMS comments
				Wistar rats.		not relevant for the EU glyphosate renewal. Kalach 360 SL contains POEA (polyethoxylated tallow amine) which is not permitted for use in formulated herbicidal products in the EU.	
68	Toxicology and metabolism	Kass L. *et al.*	2020	Relationship between agrochemical compounds and mammary gland development and breast cancer.	Molecular and cellular endocrinology, (2020) Vol. 508, Art. No. 110789	This very limited literature review focuses on the endocrine disrupting potential of different agrochemicals with glyphosate among them. Some relevant in vitro, in vivo and epidemiological studies assessing the effects of glyphosate on several factors related to endocrine function, are summarised. The cited monitoring studies highlight that glyphosate-based herbicides were detected in human samples (milk, urine, maternal blood), but contrary scientific papers are not cited. In vitro studies performed with relevant cell lines are presented to be affected by glyphosate. Further, in vivo studies investigating developmental parameters describe effects allegedly induced by glyphosate and are suggested to result from altered endocrine function. The literature review does not provide any new data. It only summarises existing data and states that it is not possible to distinguish if the effects are caused by the active substance glyphosate or additives in the formulation such as surfactants. Due to this statement and no new data the literature review has been classified as not relevant.	The RMS agrees with the applicant's justification.

Submission Number	Technical section	Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria)	RMS comments
69	Toxicology and metabolism	McCully K. S.	2020	Environmental Pollution, Oxidative Stress and Thioretinaco Ozonide: Effects of Glyphosate, Fluoride and Electromagnetic Fields on Mitochondrial Dysfunction in Carcinogenesis, Atherogenesis and Aging.	Annals of clinical and laboratory science, (2020) Vol. 50, No. 3, pp. 408-411	No experimental set up is used to investigate glyphosate toxicity. Therefore, it cannot provide new information. A very limited toxicological evaluation of glyphosate fluoride and electromagnetic fields is described based on selected literature, very specific endpoints and not specifiying the compound (glyphosate as active ingredient or formulation) which was used in the studies that are cited. This review is considered not relevant.	The RMS agrees with the applicant's justification.
70	Toxicology and metabolism	Mendler A. *et al.*	2020	Mucosal-associated invariant T-Cell (MAIT) activation is altered by chlorpyrifos- and glyphosate-treated commensal gut bacteria.	Journal of Immunotoxicology, (2020) Vol. 17, No. 1, pp. 10-20	Gut microbiome (Escherichiacoli, Bifidobacterium adolescentis and Lactobacillus reuteri) were exposed to different concentrations of a glyphosate formulation. Peripheral blood mononuclear cells (PBMCs) obtained from human volunteers were then stimulated with either pesticide-treated or non-treated bacteria (B. adolescentis or L. reuteri). Untreated E. coli were added later. MAIT cells were identified with flow cytometry and riboflavin and folate contents were measured using LC-MS/MS and electroluminescence immunoassay, respectively. A proteomic analysis was performed in E. coli. In conclusion, glyphosate might alter bacterial metabolism potentially leading altered inflammatory immune responses. A formulation of glyphosate is tested in vitro. The article is excluded as in vitro testing of glyphosate formulations produce surfactant induced cytotoxicity that is not representative for glyphosate or relevant of human in vivo exposure scenarios. Further, PBMCs instead of MAIT cells derived from gut were used.	The RMS agrees with the applicant's justification.
71	Toxicology and metabolism	Namratha M. L. *et al.*	2019	Effect of glyphosate (GLP) induced toxicity on body weights and gross pathology: ameliorative effect of ascorbic acid (AA) in wistar rats	International Journal of Current Microbiology and Applied Sciences, (2019) Vol. 8, No. 10, pp. 1486-1493	Roundup® (41%) procured in India is not the EU representative formulation (the composition and surfactant system differs from the EU representative formulation), therefore the article is not relevant for the EU glyphosate renewal.	The RMS agrees with the applicant's justification.

Submission Number	Technical section	Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria)	RMS comments
72	Toxicology and metabolism	Ongono J. S. *et al.*	2020	Pesticides used in Europe and autism spectrum disorder risk: can novel exposure hypotheses be formulated beyond organophosphates, organochlorines, pyrethroids and carbamates? - A systematic review.	Environmental research, (2020) Vol. 187, pp. 109646	Review on potential role of neuro- and thyrotoxic pesticides authorized in Europe other than those widely studied (i.e. OCs, OPs, pyrethroids and carbamates) in the risk of ASD in children or ASD behavioral phenotypes in rodents. This publication is very theoretical and it relies on the interpretation of anxiety like behaviour seen in mice (but not in rats) and the extrapolation of this to humans. Also the paper only identifies a "potential link", there is no actual exposure driven observations in humans. Therefore the publication is considered as not relevant.	The RMS agrees with the applicant's justification.
73	Toxicology and metabolism	Onyekachi U. C. *et al.*	2019	Chemoprotective potentials of selected dietary supplements in glyphosate-based herbicide-induced nephrotoxicity and dyslipidemia in albino wistar rats	Asian Journal of Biological Sciences, (2019) Vol. 12, No. 2, pp. 320-327	Intraperitoneal injection with formulated product containing surfactant is not relevant to real life exposure scenarios. Environmental fate, metabolism and pharmaco-kinetics for glyphosate active ingredient versus sufractants are very different. Given the direct systemic exposure, these data are considered irrelevant to livestock and human health risk assessments.	The RMS agrees with the applicant's justification.
74	Toxicology and metabolism	Pu Y. *et al.*	2020	Glyphosate exposure exacerbates the dopaminergic neurotoxicity in the mouse brain after repeated administration of MPTP.	Neuroscience letters, (2020) Vol. 730, Art. No. 135032	This in vivo study investigated the potential of Roundup Maxload to cause parkinson's disease. Roundup Maxload (48% w/v, potassium salt), 52% other ingredients such as water and surfactants), is not the EU representative formulation, therefore the article is not relevant for the the EU glyphosate renewal.	The RMS agrees with the applicant's justification.
75	Toxicology and metabolism	Pu Y. *et al.*	2020	Maternal glyphosate exposure causes autism-like behaviors in offspring through increased expression of soluble epoxide hydrolase.	Proceedings of the National Academy of Sciences of the United States of America, (2020) Vol. 117, No. 21, pp. 11753-11759	In this in vivo study one concentration of Roundup Maxload was used to dose pregnant mice in drinking water to investigate the risk for autism spectrum disorder in their offspring. Roundup Maxload (48% w/v, potassium salt, 52% other ingredients such as water and surfactants), is not the EU representative formulation, therefore the article is not relevant for the EU glyphosate renewal.	The RMS agrees with the applicant's justification.
76	Toxicology and metabolism	Schnabel K. *et al.*	2020	Functionality and DNA-damage properties of blood cells in lactating cows exposed to glyphosate	Archives of animal nutrition, (2020) Vol. 74, No. 2, pp. 87-106	The formulation tested is not the EU representative formulation MON 52276. Roundup Record (007525-60/MOT), Monsanto, Agrar Deutschland GmbH	The RMS agrees with the applicant's justification.

Submission Number	Technical section	Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria)	RMS comments
				contaminated feed at different feed energy levels.		(Düsseldorf, Germany) was used as water-soluble granulate, containing 720 g GL/kg as an active ingredient. Therefore te article is not relevant for the EU glyphosate renewal.	
77	Toxicology and metabolism	Turkmen R. *et al.*	2020	Determination of acute oral toxicity of glyphosate isopropylamine salt in rats.	Environmental science and pollution research international, (2020) Vol. 27, No. 16, pp. 19298-19303	The glyphosate formulation Knockdown 48 SL, which is marketed by Safa Tarım Inc. in Turkey, is not the EU representative formulation therefore the publication is not relevant for the EU glyphosate renewal.	The RMS agrees with the applicant's justification.
78	Toxicology and metabolism	Zhao J. B. *et al.*	2020	Clinical analysis of 15 cases of acute glufosinate poisoning.	Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chinese journal of industrial hygiene and occupational diseases, (2020) Vol. 38, No. 5, pp. 372-374	This publication discusses acute poisoning cases of glufosinate-ammonium. Glyphosate was mentioned only once in the following context: Glufosinate is a broadspectrum contact herbicide. Its toxicity is between glyphosate and paraquat.	The RMS agrees with the applicant's justification.

Endocrine disruption top up literature search (Nov 2016 to July 2019)

A separate literature search was conducted for relevant publications on endocrine disruption (KCA 5.8.3-019).

1. Summary ED literature search

The objective of this Endocrine Disruptor (ED)-specific literature search was to ascertain whether any scientific peer-reviewed open literature would address the potential endocrine-disrupting properties of glyphosate.
As the previous endocrine disruptor literature search, already evaluated at EU level during the previous assessment, covers the publication period between January 2014 and October 2016 a new search was conducted to cover the period from November 2016 to July 2019.

2. Search strategy ED

2.1 Data of the search ED

The search was conducted on 10 August 2019

2.2 Time window of the literature search ED

The search covers the publication period between **November 2016 and July 2019.**

2.3 Bibliographic Databases used in the literature review ED

An overview of the databases used is provided in the tables below:

Table 1: Bibliographic databases used in the literature review

Data requirement(s) captured in the search	Details of the search			
	1. AGRICOLA	2. BIOSIS	3. CABA	4. CAPLUS
Justification for choosing the source:	Provides literature from agriculture and related fields, e.g. biology, biotechnology, botany, ecology etc.	Provides the most comprehensive and largest life science database for literature, e.g. biosciences, biomedicine etc.	Provides literature from agriculture and related sciences, e.g. biotechnology, forestry, veterinary medicine etc.	Provides literature from chemistry and related fields, e.g. biochemistry chemical engineering etc.
Number of records in the database at the time of search:	> 6.1 million (05/2018)	> 27.8 million (04/2019)	> 8.9 million (05/2018)	> 48.7 million (11/2017)
Database update:	Monthly	Weekly	Weekly	Daily updates with biblio. data; weekly updates with indexing data
Date of the search:	10 Aug 2019	10 Aug 2019	10 Aug 2019	10 Aug 2019
Database covers records:	1970-present	1926-present	1973-present	1907-present and more than 180,00 pre-1907
Date of the latest database update:	5 Aug 2019	14 Aug 2019	14 Aug 2019	18 Aug 2019
Language limit:	No	No	No	No
Document types excluded that are not "scientific peer-reviewed open literature":	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE
Search strategy:	Details are listed in below.			
Total number of records retrieved:	1284	1445	1702	1527

Data requirement(s) captured in the search	Details of the search		
	5. MEDLINE	6. EMBASE	7. TOXCENTER
Justification for choosing the source:	Provides literature from every area of medicine	Provides literature from biomedicinal and pharmaceutical fields, e.g. bioscience, biochemistry, human medicine, forensic science paediatrics, pharmacy, pharmacology, drug therapy, psychiatry, public health, biomedical engineering, environmental science.	Provides literature on pharmacological, biochemical, physiological, and toxicological effects of drugs and other chemicals.
Number of records in the database at the time of search:	> 28.7 million (08/2018)	> 34.3 million (08/2018)	> 13.6 million (08/2018)
Database update:	Six times each week, with an annual reload	Daily	Weekly
Date of the search:	10 Aug 2019	10 Aug 2019	10 Aug 2019
Database covers records:	1946-present	1974-present	1907-present
Date of the latest database update:	18 Aug 2019	16 Aug 2019	12 Aug 2019
Language limit:	No	No	No
Document types excluded that are not "scientific peer-reviewed open literature":	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE
Search strategy:	Details are listed below.		
Total number of records retrieved:	894	738	1886

Data requirement(s) captured in the search	Details of the search			
	8. FSTA	9. PQSCITECH	10. ESBIOBASE	11. SCISEARCH
Justification for choosing the source:	Provides literature on scientific and technological aspects of the processing and manufacture of human food products, e.g. biotechnology, hygiene and toxicology, engineering etc.	Provides a valuable and huge resource of literature (merge of 25 STN databases) from all science areas and technology; from engineering to lifescience.	Provides comprehensive literature c the entire spectrum of biological an biosciences research, e.g. microbiology, biotechnology, ecological & environmental science genetics, plant and crop science, toxicology and many more.	Provides one of the largest multidisciplinary scientific literatur databases covering a broad field of sciences, technology, and biomedicine.
Number of records in the database at the time of search:	> 1.4 million (07/2018)	> 32 million (07/2017);	> 7.6 million (07/2018)	> 45 million (08/2018)
Database update:	Weekly	Monthly	Weekly	Weekly
Date of the search:	10 Aug 2019	10 Aug 2019	10 Aug 2019	10 Aug 2019
Database covers records:	1969-present	1962-present	1994-present	1974-present
Date of the latest database update:	15 Aug 2019	23 Jul 2019	15 Aug 2019	12 Aug 2019
Language limit:	No	No	No	No
Document types excluded that are not "scientific peer-reviewed open literature":	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE	COMMENT? or DISSERTATION or EDITORIAL or MEETING? or NEWS? or PATENT or PRESS RELEASE
Search strategy:	Details are listed in below.			
Total number of records retrieved:	105	610	988	2067

The databases used are considered to be acceptable.

2.4 Input parameters for literature search ED:

Input parameters glyphosate

Substance name	Glyphosate Salts: isopropylamine, potassium, ammonium, methylmethanamine
IUPAC/CA name	2-(phosphonomethylamino)acetic acid
CAS number(s)	1071-83-6 Salts: 38641-94-0, 70901-12-1, 39600-42-5, 69200-57-3, 34494-04-7, 114370-14-8, 40465-66-5, 69254-40-6

2.5 Endpoint specific search terms

The following specific search terms were used:

Table 1: Keywords used for the active substance glyphosate

Gly1: Glyphosate	glyphosat? OR glifosat? OR glyfosat? OR N phosphonomethyl glycine OR phosphonomethyl amino acetic acid OR 1071-83-6 OR 38641-94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR 40465-66-5 OR 69254-40-6

2.6 Filters ED

Endpoint specific search filters were applied as indicated below.

Table 2 Search filter related to the technical section toxicology

Toxicology
Gly1 AND the following search filters
TOXI? OR HAZARD OR ADVERSE OR HEALTH OR EFFECT OR NOAEL OR NOEL OR LOAEL OR LOEL OR BMD OR IN VIVO OR IN VITRO OR ACUTE OR SUBACUTE OR SUBCHRONIC OR CHRONIC OR ORAL OR DERMAL OR GAVAGE OR DIET? OR INHAL? OR RAT OR DOG OR RABBIT OR GUINEA PIG OR MOUSE OR MICE OR HAMSTER OR METABOLISM OR METABOLITE OR METABOLIC OR DISTRIBUTION OR ADSORPTION OR EXCRETION OR ELIMINATION OR KINETIC OR PBPK OR CYP OR CYTOCHROME OR ENZYM? OR GEN? OR MUTA? OR CHROMOS? OR CLASTOGEN? OR DNA OR CARCINO? OR CANCER? OR IMMUN? OR NEUR? OR BEHAV? OR ENDOCRIN? OR HORMON? OR REPRODUCT? OR DEVELOPMENT? OR MALFORMATION? OR ANOMAL? OR FERTIL? OR FOET? OR FETO? OR FETUS OR MATERN? OR PREGNAN? OR EMBRYO? OR EPIDEM? OR MEDICAL? OR POISON? OR ESTROGEN? OR ANDROGEN? OR STEROIDOGEN? OR ESTROGEN RECEPTOR? OR ANDROGEN RECEPTOR? OR THYROID OR RECEPTOR? OR AR BIND? OR ER BIND? OR THYROID OR STIMULATING HORMONE OR TSH OR LUTEINIZING HORMONE OR LH(5A)HORM OR ONE OR FOLLICULE STIMULATING HORMONE OR FSH OR ESTRADIOL? OR TESTOSTERONE? OR ACCESSORY GLAND? OR GENITAL? OR COAGULATING OR GLAND? OR PROSTATE? OR TESTIS? OR MOTILITY? OR MAMMARY GLAND? OR CERVIX? OR UTERUS? OR OVARY? OR ANOGENITAL? OR NIPPLE RETENTION? OR PREPUTIAL? OR VAGINA? OR ESTRUS? OR FOLLICULAR? OR ENDOCRINE? OR ENDOCRINE DISRUPT? OR DEVELOPMENTAL? OR REPROT? OR SEXUAL MATURATION? OR OECD 407 OR OECD 408 OR OECD 414 OR OECD 415 OR OECD 421 OR OECD 422 OR OECD 426 OR OECD 451 OR OECD 453 OR OECD 416 OR OECD 443 OR OPPTS 890.1500# OR OPPTS OR 890.1450#

3. Search results ED

Total number of hits from all search:	5036
Total number of hits after removal of duplicates:	4024

4. Evaluation ED

4.1 Rapid assessment ED literature

For the rapid assessment articles were evaluated at title and abstract level. Articles that were clearly to non-ED topics such as chemical synthesis, efficacy or analytical methods were excluded (1466 publications). In addition, 2194 publications were excluded that were related to compounds other than glyphosate or were related to non-ED effects such as acute toxicity.

4.2 Detailed assessment ED literature

After the rapid assessment 384 publication (for both toxicology and ecotoxicology) were considered relevant for detailed assessment based on the full text.

The criteria of relevance that were applied were as followed:

***In vivo* toxicology studies**	***In vitro* toxicology studies**
Exposure to glyphosate acid or its salts only	Exposure to glyphosate acid or its salts only
Use of relevant route of administration	Use of relevant system
Use of relevant test species	Test concentration in physiological acceptable range (< 1 mM)
Adequate description of study results	Adequate description of study results
Exposure to glyphosate containing formulations	Exposure to glyphosate containing formulations
Adequate description of exposure circumstances	Adequate description of exposure circumstances
Adequate description of sampling	Adequate description of study population
Adequate description of method of analysis	Adequate description of epidemiological method followed.

Of the 384 articles 347 were concluded to be non-relevant.

Of the 47 publications concluded to be relevant 17 were included in the dossier. 30 publications were concluded to be non-reliable.

The applicant provided the following tabular summary of the literature review:

	Number	**Justification**
Total number of summary records retrieved from search.	5036	n.a
Total number of summary records retrieved after removing duplicates from all database searches. [a)]	4024	n.a.
Number of summary records excluded after rapid assessment for relevance (by title / abstract).	3640	Irrelevant information for the assessment of the ED potential of Glyphosate
Total number of full-text documents assessed in detail.	384	n.a.
Number of studies excluded from the risk assessment after detailed assessment of full-text documents (*i.e.* not relevant).	347	See table B.6.10-7
Number of articles not excluded after detailed assessment. [c)]	47 (37 + 10[d)]	See table B.6.10-6
Number of articles / summaries presented in the dossier. [e)]	17	See table B.6.10-5

[a)] Automatic and manual removal within databases.

[b)] EFSA GD category 5.4.1 a, b and c.

[c)] All articles belonging to the category A, B, C of the Point 5.4.1 (as stated in the EFSA Guidance Document).

[d)] Publication identified as being relevant for ED assessment in the literature assessment according to EFSA Guidance "Submission of of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation(EC) No 1107/2009",
EFSA Journal 2011;9(2):2092

[e)] Summaries presented in the dossier: articles classified as relevant (EFSA GD, Point 5.4.1, category A) & reliable or relevant (EFSA GD, Point 5.4.1, category A) & reliable with restrictions.

The following reliability criteria have been applied:

Reliability criteria for toxicology – epidemiology and exposure studies

Reliability criteria - toxicology	
Epidemiology studies	**Exposure studies**
Guideline-specific	**Guideline-specific**
Study in accordance to valid internationally accepted	Study in accordance to valid internationally accepted

testing guidelines/practices	testing guidelines/practices
Study completely described and conducted following scientifically acceptable standards	Study performed according to GLP
	Study completely described and conducted following scientifically acceptable standards
Test substance	**Test substance**
Exposure to formulations with only glyphosate as a.i.	Exposure to formulations with only glyphosate as a.i.
Exposure to formulations with glyphosate combined with other a.i.	Exposure to formulations with glyphosate combined with other a.i.
Exposure to various formulations of pesticides	Exposure to various formulations of pesticides
Study	**Study**
Study design – epidemiological method followed	Study design clearly described
Description of population investigated	Population investigated sufficiently described
Description of exposure circumstances	Exposure circumstances sufficiently described
Description of results	Sampling scheme sufficiently documented
Have confounding factors been considered	Analytical method described in detail
Statistical analysis	Validation of analytical method reported
	Monitoring results reported
Overall assessment: Reliable / Reliable with restrictions / Not reliable	

Reliability criteria for toxicology – *in vitro* and *in vivo* studies

Reliability criteria – toxicology and metabolism	
***In vitro* studies**	***In vivo* studies**
Guideline-specific	**Guideline-specific**
Study in accordance to valid internationally accepted testing guidelines	Study in accordance to valid internationally accepted testing guidelines.
Study performed according to GLP	Study performed according to GLP
Study completely described and conducted following scientifically acceptable standards	Study completely described and conducted following scientifically acceptable standards
Test substance	**Test substance**
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)
Only glyphosate acid or one of its salts is the tested substance	Only glyphosate acid or one of its salts is the tested substance
AMPA is the tested substance	AMPA is the tested substance
Study	**Study**
Test system clearly and completely described	Test species clearly and completely described
Test conditions clearly and completely described	Test conditions clearly and completely described

Metabolic activation system clearly and completely described	Route and mode of administration described
Test concentrations in physiologically acceptable range (< 1 mM)	Dose levels reported
Cytotoxicity tests reported	Number of animals used per dose level reported
Positive and negative controls	Method of analysis described for analysis test media
Complete reporting of effects observed	Validation of the analytical method
Statistical methods described	Analytical verifications of test media
Historical negative and positive control data reported	Complete reporting of effects observed
Dose-effect relationship reported	Statistical methods described
	Historical control data of the laboratory reported
	Dose-effect relationship reported
Overall assessment: Reliable / Reliable with restrictions / Not reliable	

A full list was provided by the applicant for the 384 articles with justification why these articles were excluded. ***These were checked by the RMS and the for the following publications additional information is requested (submit an assessment of relevance and reliability for ED endpoints):***

Author	*Year*	*Title*	*Source*
Abarikwu S. O. et al.	***2015***	***Combined effects of repeated administration of Bretmont Wipeout (glyphosate) and Ultrazin (atrazine) on testosterone, oxidative stress and sperm quality of Wistar rats.***	***Toxicology mechanisms and methods (2015), Vol. 25, No. 1, pp. 70-80***
Avila-Vazquez M. et al.	***2015***	***Cancer and detrimental reproductive effects in an Argentine agricultural community environmentally exposed to glyphosate***	***Journal of Biological Physics and Chemistry, (2015) Vol. 15, No. 3, pp. 97-110.***
Bernieri T. et al.	***2019***	***Occupational exposure to pesticides and thyroid function in Brazilian soybean farmers.***	***Chemosphere, (2019) pp. 425-429***
Parvez S. et al.	***2018***	***Glyphosate exposure in pregnancy and shortened gestational length: a prospective Indiana birth cohort study***	***Environmental Health, (2018) Vol. 17, pp. 23***
Owagboriaye F. et al.	***2019***	***Comparative studies on endogenic stress hormones, antioxidant, biochemical and hematological status of metabolic disturbance in albino rat exposed to roundup herbicide and its active ingredient glyphosate.***	***Environmental science and pollution research international, (2019) Vol. 26, No. 14, pp. 14502-14512***
Kass L. et al.	***2020***	***Relationship between agrochemical compounds and mammary gland development and breast cancer.***	***Molecular and cellular endocrinology, (2020) Vol. 508, Art. No. 110789***
George A. et al.	***2018***	***The effect of glyphosate on human sperm motility and sperm DNA fragmentation***	***International Journal of Environmental Research and Public Health (2018) Vol. 15, 1117***
Santos R. et al.	***2019***	***Thyroid and reproductive hormones in relation to pesticide use in an agricultural population in Southern Brazil.***	***Environmental Research, (2019) pp. 221-231***
Stur E. et al.	***2019***	***Glyphosate-based herbicides at low doses affect canonical pathways in estrogen positive and***	***PloS one (2019), Vol. 14, No. 7, pp. e0219610***

		negative breast cancer cell lines.	

The reason for considering these studies to be supplemental was often related to reliability issues with the study. While this might be acceptable a full evaluation by the RMS is required to confirm this conclusion. ***To allow for a transparent evaluation the applicant is requested to submit the studies listed in the table above including study summaries and an evaluation.***

Table B.6.10-5: Overview of relevant articles included in the ED WoE assessment by the applicant (category A; relevant and reliable or reliable with restrictions after detailed assessment) - *ED top up literature search*

SANCO datapoint (KCA)	Autor	Year	Title	Source	Classification according to EFSA Guidance 2092Point 5.4.1	RMS
KCA 5.6	Dai P. et al.	2016	Effect of glyphosate on reproductiveorgans in male rat.	Acta Histochemica 118 (2016) 519–526	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoEassessment.	Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Forgacs A. et al.	2012	BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmenta toxicants.	TOXICOLOGICAL SCIENCES 127 (2), 391–402 (2012)	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Johansson H. et al	2018	Exposure to a glyphosate-based herbicide formulation, but not glyphosate alone, has only minor effects on adult rat testis.	Reproductive toxicology(2018) Vol. 82, pp. 25-31	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoEassessment.	Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Manservisi F. etal.	2019	The Ramazzini Institute 13-week pilot study glyphosate-based herbicides administered a human-equivalent dose to Sprague Dawley rats effects on development and endocrine system.	Environmental health : a global access science source, (2019) Vol18, No. 1, pp 15	5.4.1 case a) relevant and provides data for the riskassessment: Summary is provide in the ED WoE assessment.	Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Panzacchi S. et al.	2018	The Ramazzini Institute 13-week study on glyphosate-based herbicides at humanequivalent dose in Sprague Dawlerats: study design and first in-life endpoints evaluation	Environmental Health, (2018) Vol. 17, pp. 52	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Perego M. et al.	2016	Evidence for direct effects of glyphosate on ovarian function: glyphosate influences steroidogenesis and proliferation of bovine granulosa but not theca cells in vitro.	Journal of applied toxicology (2017) Vol. 37, No. 6, pp. 692-698	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Pham T. et al.	2019	Perinatal Exposure to Glyphosate and a Glyphosate-Based Herbicide Affect Spermatogenesis in Mice.	Toxicological sciences(2019) Vol 169 No. 1, pp. 260-271	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoEassessment	Refer to B.6.6.3.1 for an assessment of the study.

KCA 5.6	Gorga A. et al.	2019	In vitro effects of glyphosate and Roundup on Sertoli cell physiology.	Toxicology in vitro : an internationa journal published in association with BIBRA,Electronic Publication Date: 15 Oct 2019 Journal code: 8712158. E-ISSN: 1879-3177. L-ISSN: 0887-2333.	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Zhang J. et al.	2019	The toxic effects and possible mechanisms of glyphosate on mouse oocytes.	Chemosphere, (2019) Vol. 237, pp. 124435	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.8.3	Brennan JC. et al.	2016	Development of a recombinant human ovarian (BG1) cell line containing oestrogen receptor alpha and beta for improved detection of oestrogenic/ Antioestrogenic chemicals; study supplementary	Environ Toxicol Chem. (2016) 35:91-100.	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.8.3 for an assessment of the study.
KCA 5.8.3	Defarge N. et al.	2016	Coformulants in glyphosate-based herbicides disrupt aromatase activity in human cells below toxic levels; study supplementary	Int J Environ Res Public Health. 2016 Feb 26;13(3).	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.8.3 for an assessment of the study.
KCA 5.8.3	Gigante P. et al.	2018	Glyphosate affects swine ovarian and adipose stromal cell functions.	Animal reproduction science, (2018) Vol. 195, pp. 185-196	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.8.3 for an assessment of the study.
KCA 5.8.3	Martina B. et al.	2019	Quizalofop-p-Ethyl Induces Adipogenesi in 3T3-L1 Adipocytes	TOXICOLOGICAL SCIENCES, 170 (2019), 452–461	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.5 (cited as Biserni *et al* 2019) for an assessment of the study.
KCA 5.8.3	Mesnage R. et al.	2017	Evaluation of estrogen receptor alpha activation by glyphosate-based herbicide constituents.	Food and Chemical Toxicology 108 (2017) 30-42	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.8.3 for an assessment of the study.
KCA 5.8.3	Thongprakaisang S. et al.	2013	Glyphosate induces human breast cancer cells growth via estrogen receptors.	Food and chemical toxicology : a international journal published fo the British Industrial Biological Research Association, (2013) Vol 59, pp. 129-36.	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.8.3 for an assessment of the study.
KCA 5.8.3	Vanlaeys A. et al.	2018	Formulants of glyphosate-based herbicides have more deleterious impact than glyphosate on TM4 Sertoli cells.	Toxicology in vitro : an internationa journal published in association with BIBRA, (2018) Vol. 52, pp. 14-22	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment.	Refer to B.6.8.3 for an assessment of the study.

Table B.6.10-6: Overview of relevant articles (based on list of all articles belonging to the category A, B, C of the Point 5.4.1 (as stated in the EFSA Guidance Document)) which are excluded* from the ED assessment - ED top up literature search

* the data in this table was provided by the applicant. The RMS has maintained the tables as submitted by the applicant for transparency reasons. However, the RMS notes that several open-literature studies were not excluded from the ED assessment, but were actually included (i.e. the studies that are listed in Table B.6.10-5). For these studies, the RMS has added a reference to the respective section where the study was evaluated in the RMS commenting box in the table below.

KCA Datapoint	Author(s)	Year	Title	Source	Classification according to EFSA Guidance 2092Point 5.4.1	RMS comment in relation to ED endpoints *For general literature review comments by RMS, please refer to open-literature study results in sections above*
KCA 5.8.3	Kongtip P. et al.	2019	Thyroid Hormones in Conventional and Organic Farmers in Thailand.	International journal of environmental research and public health, (2019) Vol. 16, No. 15. Electronic Publication Date: 29 Jul 2019	5.4.1 case b) Relevant but supplementary information. This publication is considered relevant but supplementary for the risk assessment of glyphosate due to the higher incidence of thyroid disease in women (more numerous in organic farming), no data on the menopausal status of the women (change in thyroid hormones), the collection of data with diaries of the farmers may be incomplete, the exposure of farmers to pesticides prior to the study and prior to starting organic farming, and the results for glyphosate should have been examined for confounding from other pesticides that were correlated with glyphosate use. Moreover, the userate and bioavailability (Acquavella et al. (2004) Environmental Health Perspectives Vol. 112(3), 321-326; Acquavella et al. (2006) Epidemiology, Vol. 17(1) 69-74) of glyphosate was lower than that of the other pesticides used. Since the determination of serum thyroid hormone levels is key in this study, the method of analysis should have been better documented.	The RMS agrees with the applicant's justification.

KCA 5.8.3	Parvez S. et al.	2018	Glyphosate exposure in pregnancy and shortened gestational length: prospective Indiana birth cohort study	Environmental Health, (2018) Vol. 17, pp. 23	5.4.1 case b) Relevant but supplementary information. This publication is considered relevant but supplementary for the risk assessment of glyphosate as the number of pregnancies followed was too low and n data are available on urinary concentrations of other pesticides and environmental chemicals.	**Justification not agreed by RMS. The applicant is requested to submit an assessment of relevance and reliability for ED endpoints.**
KCA 5.8.3	Pinto L. et al.	2018	Identification of candidate reference chemicals for in vitro steroidogenesis assays	Toxicology In Vitro, (2018) Vol. 47, pp. 103-119	5.4.1 case b) Relevant but supplementary information. Relevant secondary information	The RMS agrees with the applicant's justification.

KCA 5.8.3	Rappazzo K. et al	2019	Maternal residential exposure to specific agricultural pesticide active ingredients and birth defect in a 2003-2005 North Carolina birth cohort.	Birth defects research, (2019) Vol. 111, No. 6, pp. 312-323	5.4.1 case b) Relevant but supplementary information This publication is considered relevant but supplementary for the risk assessment of glyphosate because of the small number of specific birth defects. Although confounding by co-occurring pesticides has been considered in this study the potential for confounding by joint pesticide exposures and other environmental factors cannot be excluded.	Publication included in the dossier. Please refer to Volume 3 CA Appendix to B.6.6 for an assessment.
KCA 5.8.3	Shrestha S. et al.	2018	Incident thyroid disease in female spouses of private pesticide applicators.	Environment International, (September 2018) Vol. 118, pp. 282-292	5.4.1 case b) Relevant but supplementary information. Not a glyphosate specific study.	The RMS agrees with the applicant's justification.

KCA 5.8.3	Shrestha S. et al.	2018	Pesticide use and incident hypothyroidism in pesticide applicators in the agricultural health study	Environmental Health Perspectives (2018) Volume 126, Number 9, 11 p.	5.4.1 case b) Relevant but supplementary information. Not a glyphosate specific study. Self-reporting had inconsistencies at age of diagnosis, moreover only 32% of self-reported disease was confirmed by medical records.	The RMS agrees with the applicant's justification.
KCA 5.3	Owagboriaye F. e al.	2019	Comparative studies on endogenic stress hormones, antioxidant, biochemical and hematological status of metabolic disturbance in albino rat exposed to roundup herbicide and its active ingredient glyphosate.	Environmental science and pollution research international, (2019) Vol. 26, No. 14, pp. 14502-14512	5.4.1 case b) Relevant but supplementary information Considering this study represent a repeated-exposure, 90-day study dosing, several deviations can be identified from the guidance document: 1. no proper identification of the test substance presented 2. no proper description of the animals, housing conditions are reported 3. number of animals used in the dose groups is less than suggested 4. no pathology were performed 5. no historical control data mentioned 6. no clinical findings or body weight measurements recorded.	**Justification not agreed by RMS. The applicant is requested to submit an assessment of relevance and reliability for ED endpoints**

KCA 5.4	De Almeida L. et al.	2018	Moderate levels of glyphosate and its formulations vary in their cytotoxicity and genotoxicity in a whole blood model and in human cell lines with different estrogen receptor status	3 BIOTECH, (2018) Vol. 8, No. 10. ISSN: 2190-572X.	5.4.1 case b) Relevant but supplementary information. Mixed study design: in the Comet assay only 2 doses were established.	The RMS agrees with the applicant's justification.
KCA 5.4	Defarge N. et al.	2018	Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides.	Toxicology reports, (2018) Vol. 5, pp. 156-163	5.4.1 case b) Relevant but supplementary information Article is not glyphosate specific. Concentration groups are not properly described, moreover the chosen concentrations are high for in vitro studies and not applicable to test aromatase inhibition.	The RMS agrees with the applicant's justification.

KCA 5.6	Dai P. et al.	2016	Effect of glyphosate on reproductive organs in male rat.	Acta Histochemica 118 (2016) 519–526	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Forgacs A. et al.	2012	BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants.	TOXICOLOGICAL SCIENCES 127 (2) 391–402 (2012)	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Refer to B.6.6.3.1 for an assessment of the study.

KCA 5.6	Johansson H. et al	2018	Exposure to a glyphosate-based herbicide formulation, but not glyphosate alone, has only minor effects on adult rat testis.	Reproductive toxicology(2018) Vol. 82, pp. 25-31	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.6 for an assessment.
KCA 5.6	Manservisi F. et al.	2019	The Ramazzini Institute 13-week pilot study glyphosate-based herbicides administered at human equivalent dose to Sprague Dawley rats: effects on development and endocrine system.	Environmental health : a global access science source, (2019) Vol. 18, No. 1, pp 15	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Panzacchi S. et al.	2018	The Ramazzini Institute 13-week study on glyphosate-based herbicides at humanequivalent dose in Sprague Dawley rats: study design and first in-life endpoints evaluation	Environmental Health, (2018) Vol. 17, pp. 52	5.4.1 case a) relevant and provides data for the riskassessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Refer to B.6.6.3.1 for an assessment of the study.

KCA 5.6	Perego M. et al.	2016	Evidence for direct effects of glyphosate on ovarian function: glyphosate influences steroidogenesis and proliferation of bovine granulosa but not theca cells in vitro.	Journal of applied toxicology (2017) Vol37, No. 6, pp. 692-698	5.4.1 case a) relevant and provides data for the riskassessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.6 for an assessment.
KCA 5.6	Pham T. et al.	2019	Perinatal Exposure to Glyphosate and a Glyphosate-Based Herbicid Affect Spermatogenesis in Mice.	Toxicological sciences(2019) Vol. 169,No. 1, pp. 260-271	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Refer to B.6.6.3.1 for an assessment of the study.
KCA 5.6	Ren X. et al.	2018	Effects of glyphosate on the ovarian function of pregnant micethe secretion of hormones and the sex ratio of their fetuses.	Environmental pollution (2018) Vol. 243No. Pt B, pp. 833-841	5.4.1 case b) Relevant but supplementary information. Substance was not sufficiently characterized, moreover only one dose was considered as glyphosate and as RoundUp exposure group.	Publication included in the dossier. Refer to B.6.8.3 for an assessment of the study.
KCA 5.6	Sritana N. et al.	2018	Glyphosate induces growth of estrogen receptor alpha positive cholangiocarcinoma cells via non-genomic estrogen receptor/ERK1/2 signaling pathway.	Food and chemical toxicology (2018)Vol. 118, pp. 595-607	5.4.1 case b) Relevant but supplementary information. No replicates had been used in the study, the incubationtime was an extended 40 h, the active substance identification is not entirely complete, moreover the cel line selection is not explained.	Publication included in the dossier. Refer to B.6.8.3 for an assessment of the study.
KCA 5.6	Wrobel M. et al.	2018	Glyphosate affects the secretion o regulators of uterine contractions in cows while it does not directly impair the motoric function of myometrium in vitro.	Toxicology and applied pharmacology,(2018) Vol. 349, pp. 55-61	5.4.1 case b) Relevant but supplementary information This publication is considered relevant but supplemental for the risk assessment of glyphosate as the glyphosate used is not sufficiently characterized and the analysis of glyphosate, hormones and prostaglandins is not sufficiently documented.	The RMS agrees with the applicant's justification.
KCA 5.6	Gorga A. et al.	2019	In vitro effects of glyphosate and Roundup on Sertoli cell physiology.	Toxicology in vitro : an international journal published in association with BIBRA,Electronic Publication Date: 15 Oct 2019 Journal code: 8712158. E-ISSN: 1879-3177. L-ISSN: 0887-2333.	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.6 for an assessment.

KCA 5.6	Zhang J. et al.	2019	The toxic effects and possible mechanisms of glyphosate on mouse oocytes.	Chemosphere, (2019) Vol. 237, pp. 124435	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.6 for an assessment.
KCA 5.7	Gallegos C. et al.	2018	Perinatal Glyphosate-Based Herbicide Exposure in Rats Alters Brain Antioxidant Status, Glutamate and Acetylcholine Metabolism and Affects Recognition Memory.	Neurotoxicity research, (2018) Vol. 34, No. 3, pp. 363-374	5.4.1 case b) Relevant but supplementary information Not relevant as only a glyphosate based herbicide was used as test material, in two established doses. As supportive information can be transferred to the general LRR.	The RMS agrees with the applicant's justification.
KCA 5.8.3	Brennan JC. et al.	2016	Development of a recombinant human ovarian (BG1) cell line containing oestrogen receptor alpha and beta for improved detection of oestrogenic/ Antioestrogenic chemicals; study supplementary	Environ Toxicol Chem. (2016) 35:91-100.	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.8.3 for an assessment.
KCA 5.8.3	Defarge N. et al.	2016	Coformulants in glyphosate-based herbicides disrupt aromatase activity in human cells below toxi levels; study supplementary	Int J Environ Res Public Health. 2016 Feb 26;13(3).	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.8.3 for an assessment.
KCA 5.8.3	George A. et al.	2018	The effect of glyphosate on huma sperm motility and sperm DNA fragmentation	International Journal of Environmental Research and Public Health (2018) Vol. 15, 1117	5.4.1 case b) Relevant but supplementary information This publication is considered relevant but supplementa as the glyphosate used is not characterized, only one tes concentration was used, no positive control was considered and the results obtained are not corroborated by in vivo regulatory reproductive toxicology studies with much higher systemic levels of glyphosate.	**Justification not agreed by RMS. The applicant is requested to submit an assessment of relevance and reliability for ED endpoints**
KCA 5.8.3	Gigante P. et al.	2018	Glyphosate affects swine ovarian and adipose stromal cell functions	Animal reproduction science, (2018) Vol 195, pp. 185-196	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.8.3 for an assessment.

KCA 5.8.3	Martina B. et al.	2019	Quizalofop-p-Ethyl Induces Adipogenesis in 3T3-L1 Adipocytes	TOXICOLOGICAL SCIENCES, 170, (2019), 452–461	5.4.1 case a) relevant and provides data for the risk assessment: This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions.	Publication included in the dossier. Please refer to Volume 3 CA B.6.5 for an assessment. Publication cited as Biserni *et al.* 2019.
KCA 5.8.3	Mesnage R. et al.	2017	Evaluation of estrogen receptor alpha activation by glyphosate-based herbicide constituents.	Food and Chemical Toxicology 108 (2017) 30-42	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.8.3 for an assessment.
KCA 5.8.3	Sakpa C. et al.	2018	Effects of glyphosate on sperm parameters and pregnancy success rate in Wistar rats.	Annals of Biomedical Sciences (2018), Volume 17, Number 2, pp. 156-164	5.4.1 case b) Relevant but supplementary information This publication is considered relevant but supplementary for the risk assessment of glyphosate because the glyphosate used is not sufficiently characterized, only two dose levels were tested and the number of animals used per dose level was too low.	Publication included in the dossier. Please refer to Volume 3 CA B.6.6 for an assessment.
KCA 5.8.3	Santos R. et al.	2019	Thyroid and reproductive hormones in relation to pesticide use in an agricultural population i Southern Brazil.	Environmental Research, (2019) pp. 221 231	5.4.1 case b) Relevant but supplementary information. Exposure related information based on biased recall data.	**Justification not agreed by RMS. The applicant is requested to submit an assessment of relevance and reliability for ED endpoints**
KCA 5.8.3	Thongprakaisang S. et al.	2013	Glyphosate induces human breast cancer cells growth via estrogen receptors.	Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, (2013) Vol. 59, pp. 129-36.	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.8.3 for an assessment.
KCA 5.8.3	Vanlaeys A. et al.	2018	Formulants of glyphosate-based herbicides have more deleterious impact than glyphosate on TM4 Sertoli cells.	Toxicology in vitro : an international journal published in association with BIBRA, (2018) Vol. 52, pp. 14-22	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provide in the ED WoE assessment	Publication included in the dossier. Please refer to Volume 3 CA B.6.8.3 for an assessment.

KCA 5.8.3	Zhao H. et al.	2018	Effects of Glyphosate on Testosterone Synthesis in Male Rats.	Asian Journal of Ecotoxicology, (2018) Vol. 13, No. 5, pp. 242-247	5.4.1 case b) Relevant but supplementary information This publication is considered relevant but supplemental for the risk assessment of glyphosate but reliable with restrictions because the reporting of the experimental conditions is not complete.	Publication included in the dossier. Refer to B.6.8.3 for an assessment of the study.

Table B.6.10-7: Overview of articles excluded after detailed assessment (i.e. not relevant for the ED assessment); based on a list of all articles belonging to the category A, B, C of the Point 5.4.1 (as stated in the EFSA Guidance Document) – ED top up literature search

Technical section	Author(s)	Year	Title	Source	Justification	RMS comment in relation to ED endpoints *For general literature review comments by RMS, please refer to open-literature study results in the sections provided above*
Toxicology	Acquavella J.et al.	2016	Glyphosate epidemiology expert pane review: a weight of evidence systematic review of the relationship between glyphosate exposure and non Hodgkin's lymphoma or multiple myeloma.	Critical reviews in toxicology, (2016 Sep) Vol. 46No. sup1, pp. 28-43	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
Toxicology	Ait B. Y. et al.	2017	Behavioral and Immunohistochemical Study of the Effects of Subchronic an Chronic Exposure to Glyphosate in Mice.	Frontiers in behavioral neuroscience, (2017) Vol. 11, pp. 146	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Formulation tested (Roundup, 486 g/L isopropylamine salt, 360 g/L a.e.) in vivo.	The RMS agrees with the applicant's justification.
Toxicology	Almeida L. L. d. et al.	2017	Effects of melatonin in rats in the initial third stage of pregnancy exposed to sub-lethal doses of herbicides.	Acta histochemica, (2017 Apr) Vol. 119, No. 3, pp. 220-227	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Formulation tested at high doses of 500 mg/kg bw/day (Roundup).	The RMS agrees with the applicant's justification.
Toxicology	Anifandis G. eal.	2017	The In Vitro Impact of the Herbicide Roundup on Human Sperm Motility and Sperm Mitochondria.	Toxics, (2017 Dec 21) Vol. 6, No. 1	Not relevant for the assessment of ED effects: Formulation tested in vitro (Roundup, not characterized)	The RMS agrees with the applicant's justification.
Toxicology	Anon.	2017	Erratum to: Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure (Environmental Health: A Global Access Science Source (2015) 14:70 DOI: 10.1186/s12940-015-0056-1).	Environmental Health: A Global Access Science Source, (23 Mar 2017) Vol. 16, No. 1. arn. 28	Not relevant for the assessment of ED effects: This is erratum to Roy_2016.	The RMS agrees with the applicant's justification.

Toxicology	Anon.	2017	Some organophosphate insecticides and herbicides.	IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (2017), Vol. 112, VII + 452 p	Not relevant for the assessment of ED effects: secondary source of infomation.	The RMS agrees with the applicant's justification.
Toxicology	Avgerinou C. et al.	2017	Occupational, dietary, and other risk factors for myelodysplastic syndrome in Western Greece.	Hematology (Amsterdam, Netherlands), (2017 Aug) Vol. 22, No. 7, pp. 419-429	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information. A case-control study with non-blinded interviewers is compromised both by potential recall bias and interviewer bias.	The RMS agrees with the applicant's justification. For non-ED related endpoints, RMS requested a study summary in order to further justify the categorization. Refer to B.6.9 for an assessment of the study.
Toxicology	Avila-Vazquez M. et al.	2015	Cancer and detrimental reproductive effects in an Argentine agricultural community environmentally exposed to glyphosate	Journal of Biological Physics and Chemistry, (2015) Vol. 15, No. 3, pp. 97-110. CODEN: JBPCAJ.	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate with relation to ED because the general population followed was exposed to multiple environmental factors making it impossible to establish a causal relationship between exposure to glyphosate and reproductive disorders.	**Justification not agreed by RMS. The applicant is requested to submit an assessment of relevance and reliability for ED endpoints.**
Toxicology	Avila-Vazquez M. et al.	2018	Environmental exposure to glyphosat and reproductive health impacts in agricultural population of Argentina.	Journal of Environmental Protection (2018), Vol. 9, Number 3, pp. 241-253	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate in relation to ED because the general population followed was exposed to multiple environmental factors making it impossible to establish a causal relationship between exposure to glyphosate and reproductive disorders.	Publication included in the dossier. Please refer to Volume 3 CA B.6.6 for an assessment.
Toxicology	Baier C. J. et al.	2017	Behavioral impairments following repeated intranasal glyphosate-based herbicide administration in mice.	Neurotoxicology and teratology, (2017 Nov) Vol. 64, pp. 63-72	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Formulation tested via intra-nasal administration.	The RMS agrees with the applicant's justification.

Toxicology	Beecham J. E. et al.	2015	The possible link between autism and glyphosate acting as glycine mimetic a review of evidence from the literature with analysis	Journal of Molecular and Genetic Medicine, (2015) Vol. 9, No. 4, pp. 1000197/1-1000197/16	Not relevant for the assessment of ED effects: This publication is considered not relevant for glyphosate risk assessment because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
Toxicology	Beranger R. et al.	2018	Multiple pesticide analysis in hair samples of pregnant French women: Results from the ELFE national birth cohort.	Environment International, (2018) Vol. 120, pp. 43-53	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate with relation to ED because glyphosate was not	The RMS agrees with the applicant's justification.
					monitored in this multi-pesticide exposure study.	
Toxicology	Bernieri T. et al.	2019	Occupational exposure to pesticides and thyroid function in Brazilian soybean farmers.	Chemosphere, (2019) pp. 425-429	Not relevant for the assessment of ED effects: This study is considered not relevant for the risk assessment of glyphosate with relation to ED because there was exposure to multiple pesticides with no quantitative estimate of exposure to glyphosate.	**Justification not agreed by RMS. The applicant is requested to submit an assessment of relevance and reliability for ED endpoints**
Toxicology	Berry C.	2013	Comments on "Long term toxicity of Roundup herbicide and a Roundup-tolerant genetically modified maize".	Food and Chemical Toxicology, (MAR 2013) Vol 53, pp. 430-431	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Letter to Editor	The RMS agrees with the applicant's justification.
Toxicology	Bhardwaj K. e al.	2019	Effective attenuation of glyphosate-induced oxidative stress and granulos cell apoptosis by vitamins C and E in caprines	Molecular reproduction and development, (2019) Vol. 86, No. 1, pp. 42-52	Not relevant for the assessment of ED effects: This publication is found not relevant for the risk assessment of glyphosate because it is not clear that either glyphosate or a glyphosate formulation was tested and the effects investigated were only found at concentrations beyond a concentration range that is physiologically possible (> 1mM).	The RMS agrees with the applicant's justification.

Toxicology	Bonvallot N. e al.	2018	Metabolome disruption of pregnant rats and their offspring resulting from repeated exposure to a pesticide mixture representative of environmental contamination in Brittany	PLoS One, (2018) Vol. 13, No. 6, pp.	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate in relation to ED because the rats in this study were orally exposed to a mixture of 8 pesticides including glyphosate.	The RMS agrees with the applicant's justification.
Toxicology	Brusick D. et al.	2016	Genotoxicity Expert Panel review: weight of evidence evaluation of the genotoxicity of glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid.	Critical reviews in toxicology, (2016 Sep) Vol. 46 No. sup1, pp. 56-74	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: review, secondary source	The RMS agrees with the applicant's justification.
Toxicology	Burstyn I. et al	2017	Visualizing the heterogeneity of effects in the analysis of associations of multiple myeloma with glyphosate use. comments on sorahan, t. multiple myeloma and glyphosate use: A re-analysis of us agricultural health study (AHS) data. int. j. environ. res. public health 2015, 12, 1548-1559.	International Journal of Environmental Research and Public Health, (January 2017) Vol. 14, No. 1. arn. 5	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Reanalysis of old data, no stastically significant glyphosate findings.	The RMS agrees with the applicant's justification.
Toxicology	Bus J. S.	2017	IARC use of oxidative stress as key mode of action characteristic for facilitating cancer classification: Glyphosate case example illustrating lack of robustness in interpretative implementation.	Regulatory toxicology and pharmacology : RTP, (2017 Jun) Vol. 86, pp. 157-166	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
Toxicology	Caganova B. e al.	2017	Caustic effects of chemicals: risk factors for complications and mortalit in acute poisoning	Monatshefte fuer Chemie, (2017) Vol. 148, No. 3, pp. 497-503	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article discusses caustic injury in suicide attempts and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
Toxicology	Caganova B. e al.	2017	Caustic ingestion in the elderly: influence of age on clinical outcome	Molecules, (2017) Vol. 22, No. 10, pp. 1726/1- 1726/11	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article compares outcomes of caustic ingestions in elderly to younger patients and unsurprisingly demonstrates that there is a higher mortality in the older group. Glyphosate is mentioned in a table where there were 9 ingestions with no fatalities in the younger group and 2 fatalities in the elderly. This article	The RMS agrees with the applicant's justification.

					discusses suicidal ingestions of caustic substances and should therefore not impact re-registration.	
Toxicology	Cai W. et al.	2017	Effects of glyphosate exposure on sperm concentration in rodents: A systematic review and meta-analysis.	Environmental toxicology and pharmacology, (2017 Oct) Vol. 55, pp. 148-155	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Re-evaluation of pooled literature data.	The RMS agrees with the applicant's justification. Refer to B.6.6.3.1 for an assessment of the study.
Toxicology	Caloni F. et al.	2018	In vitro effects of two environmental toxicants, beauvericin and glyphosate in Roundup, on cell proliferation and steroidogenesis using a novel bovine whole ovarian cell culture system	JOURNAL OF VETERINARY PHARMACOLOGY AND THERAPEUTICS, (2018) Vol. 41, Supp. [1], Sp. iss. SI, pp. 103-104	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate with relation to ED because cells in culture were exposed to a glyphosate formulation which is not appropriate because of the presence of surfactant.	The RMS agrees with the applicant's justification.
Toxicology	Caloni F. et al.	2016	Suspected poisoning of domestic animals by pesticides.	The Science of the total environment, (2016 Jan 01) Vol. 539, pp. 331-336	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Review article on domestic animal poisonings by pesticides.	The RMS agrees with the applicant's justification.
Toxicology	Camacho A. et al.	2017	The health consequences of aerial spraying illicit crops: The case of Colombia.	Journal of health economics, (2017 Jul) Vol. 54, pp. 147-160	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because it is too general and no specific epidemiological method was followed to establish an association between the application of glyphosate and disease outcome.	The RMS agrees with the applicant's justification.
Toxicology	Caramello C. S. et al.	2017	Evaluation of herbicide glyphosate effects in the fish Prochilodus lineatus using chromosome aberration test.	Revista Veterinaria (2017), Vol. 28, No. 1, pp. 65-68	Not relevant for the assessment of ED effects: Formulation tested (Roundup Full II), not representative for the renewal.	The RMS agrees with the applicant's justification.

Toxicology	Cattani D. et al	2017	Developmental exposure to glyphosate-based herbicide and depressive-like behavior in adult offspring: Implication of glutamate excitotoxicity and oxidative stress.	Toxicology, (20170715) Vol. 387, pp. 67-80	Not relevant for the assessment of ED effects: Formulation tested (Roundup Original, Brazil, 360 g/L glyphosate), not- representative for the renewal.	The RMS agrees with the applicant's justification.
Toxicology	Cattani D. et al	2014	Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity.	Toxicology, (2014 Jun 05) Vol. 320, pp. 34-45	Not relevant for the assessment of ED effects: Formulation tested (Roundup Original, Brazil, 360 g/L glyphosate), not- representative for the renewal.	The RMS agrees with the applicant's justification.
Toxicology	Cattelan M. et al.	2018	Occupational exposure to pesticides i family agriculture and the oxidative, biochemical and hematological profil in this agricultural model	Life Sciences, (2018) Vol. 203, pp. 177-183	Not relevant for the assessment of ED effects: Related to epidemiology section of dossier, but not relevant to ED.	The RMS agrees with the applicant's justification.
Toxicology	Chang E. T. et al.	2016	Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes, (2016) Vol. 51, No. 6, pp. 402-34	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: The glyphosate meta-RRs took the results from the available studies at face value. The authors had no way to correct for recall bias, confounding, etc. Therefore, the meta-RRs are in error to the extent that the studies included in the meta-analysis are in error. Chang and Delzell (2016) are clear on this point in their meta-analysis article. Accordingly, p values and confidence intervals for the meta-RRs cannot be taken at face value because they incorporate systematic error or bias. Thus, the argument about the statistical significance (or not) of the meta-RR for glyphosate is inconsequential. You cannot calculate a valid p value when there is uncontrolled systematic error (Greenland S. Randomization, statistics, and causal inference. Epidemiology 1990; 1:421-429).	RMS requested a study summary in order to further justify the categorization. Refer to B.6.9 for an assessment of the study.

Toxicology	Chlopecka M. et al.	2014	Glyphosate affects the spontaneous motoric activity of intestine at very low doses - in vitro study.	Pesticide biochemistry and physiology, (2014 Jul) Vol. 113, pp. 25-30	Not relevant for the assessment of ED effects: novel ex-vivo model not relevant to 1107/2009 review.	The RMS agrees with the applicant's justification.
Toxicology	Chlopecka M. et al.	2017	The effect of glyphosate-based herbicide Roundup and its co-formulant, POEA, on the motoric activity of rat intestine - In vitro study	Environmental toxicology and pharmacology, (2017 Jan) Vol. 49, pp. 156-162	Not relevant for the assessment of ED effects: Formulation and mixtures of glyphosate and surfactant tested in vitro (Roundup ULTRA 170 SL; 170 g isopropylamine salt/L).	The RMS agrees with the applicant's justification.
Toxicology	Clark P. A. et al.	2016	Chronic kidney disease in Nicaraguan sugarcane workers: A historical, medical, environmental analysis and ethical analysis.	Internet Journal of Third World Medicine, (2016) Vol. 12, No. 1	Not relevant for the assessment of ED effects: This publication is considered not relevant for glyphosate risk assessment because no systematic epidemiological approach was followed with no figures reported on workers observed with their exposure patterns.	The RMS agrees with the applicant's justification.
Toxicology	Clausing P.	2017	Cancer risk by glyphosate: The "Weight of Evidence Approach" of BfR. Krebsgefahr durch Glyphosat: Der "Weight of Evidence Approach" des BfR.	Umweltmedizin Hygiene Arbeitsmedizin (2017), Vol. 22, No. 1, pp. 27-34	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work.	The RMS agrees with the applicant's justification.
Toxicology	Connolly A. et al.	2017	Exposure assessment using human biomonitoring for glyphosate and fluroxypyr users in amenity horticulture.	International journal of hygiene and environmenta health, (20170800) Vol. 220, No. 6, pp. 1064-107	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	The RMS agrees with the applicant's justification.
Toxicology	Conrad A. et a	2017	Glyphosate in German adults - Time trend (2001 to 2015) of human exposure to a widely used herbicide	International journal of hygiene and environmenta health, (2017 Jan) Vol. 220, No. 1, pp. 8-16	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	The RMS agrees with the applicant's justification.
Toxicology	Coullery R. P. et al.	2016	Neuronal development and axon growth are altered by glyphosate through a WNT non-canonical signaling pathway.	Neurotoxicology, (2016 Jan) Vol. 52, pp. 150-61	Not relevant for the assessment of ED effects: High in vitro doses >10 mM	The RMS agrees with the applicant's justification.

Toxicology	Cremonese C. et al.	2017	Occupational exposure to pesticides, reproductive hormone levels and sperm quality in young Brazilian men	Reproductive Toxicology, (2017) Vol. 67, pp. 174 185	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Due to exposure/outcome temporal ambiguity and failure to control for other exposures in the evaluation of specific exposures.	The RMS agrees with the applicant's justification. For non-ED endpoints, RMS requested a study summary in order to further justify the categorization. Refer to B.6.9 for an assessment of the study.
Toxicology	Dar A. M. et al	2015	Single and interactive toxic potential of Rroundup and ammonium nitrate on Haemato-biochemical parameters in wistar rats	Journal of Cell and Tissue Research, (2015) Vol. 15, No. 3, pp. 5295-5299	Not relevant for the assessment of ED effects: High dose of glyphosate based herbicide administered to rats in drinking water.	The RMS agrees with the applicant's justification.
Toxicology	de Araujo J. et al.	2016	Glyphosate and adverse pregnancy outcomes, a systematic review of observational studies.	BMC public health, (20160606) Vol. 16, pp. 472	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Review of literature	The RMS agrees with the applicant's justification.
Toxicology	de Aguiar L.. e al.	2016	Glyphosate-based herbicide exposure causes antioxidant defence responses in the fruit fly Drosophila melanogaster.	Comparative biochemistry and physiology. Toxicology & pharmacology : CBP, (2016 Jul-Aug) Vol. 185-186, pp. 94-101	Not relevant for the assessment of ED effects: Tested formulation (Roundup Original) for cellular mechanisms in houseflies, not directly relevant to human health risk assessment.	The RMS agrees with the applicant's justification.
Toxicology	de Avila Renato I. et al.	2017	In vitro assessment of skin sensitization, photosensitization and phototoxicity potential of commercial glyphosate-containing formulations.	Toxicology in vitro : an international journal published in association with BIBRA, (2017 Dec) Vol. 45, No. Pt 3, pp. 386-392	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Non-validated model confirms glyphosate non-sensitized and is a non-photosensitizer. Formulation data inconsistent in non- validated model.	The RMS agrees with the applicant's justification.
Toxicology	de Moura F. et al.	2017	Effects of glyphosate-based herbicide on pintado da Amazonia: Hematology histological aspects, metabolic parameters and genotoxic potential.	Environmental toxicology and pharmacology, (2017 Dec) Vol. 56, pp. 241-248	Not relevant for the assessment of ED effects: high doses of glyphosate based herbicide to aquatic species.	The RMS agrees with the applicant's justification.

Toxicology	de Souza. J. et al.	2017	Perinatal exposure to glyphosate-based herbicide alters the thyrotrophic axis and causes thyroid hormone homeostasis imbalance in male rats.	Toxicology, (20170215) Vol. 377, pp. 25-37	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because a glyphosate formulation (Roundup Transorb) was used instead of glyphosate.	The RMS agrees with the applicant's justification.
Toxicology	Dechartes J. et al.	2019	Glyphosate and glyphosate-based herbicide exposure during the peripartum period affects maternal brain plasticity, maternal behaviour and microbiome	Journal of Neuroendocrinology, (2019) pp. Ahead of Print. CODEN: JOUNE2. ISSN: 0953-8194.	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate in relation to ED as no effects were studied related to endocrine organs.	The RMS agrees with the applicant's justification.
Toxicology	Dedeke Gabrie A. et al.	2018	Comparative Assessment on Mechanism Underlying Renal Toxicity of Commercial Formulation of Roundup Herbicide and Glyphosat Alone in Male Albino Rat.	International journal of toxicology, (2018) Vol. 37 No. 4, pp. 285-295	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate with relation to ED as no ED related endpoints were investigated in this study.	The RMS agrees with the applicant's justification.
Toxicology	Defarge N. et al.	2016	Co-Formulants in Glyphosate-Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels.	International journal of environmental research an public health, (2016 Feb 26) Vol. 13, No. 3	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: In vitro results not significant for glyphosate vs multiple formulations or mixtures.	The RMS agrees with the applicant's justification.
Toxicology	Diken M. E. et al.	2017	In vitro effects of some pesticides on glutathione-s transferase activity.	Fresenius Environmental Bulletin (2017), Vol. 26, No. 12A, pp. 8023-8029	Not relevant for the assessment of ED effects: excessively high in vitro doses in the mM range.	The RMS agrees with the applicant's justification.
Toxicology	Dung Le Tien et al.	2013	Comments on "Long term toxicity of Roundup herbicide and a Roundup-tolerant genetically modified maize".	Food and Chemical Toxicology, (MAR 2013) Vol 53, pp. 428-429	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Letter to Editor	The RMS agrees with the applicant's justification.
Toxicology	Fagan J. et al.	2015	The Seralini affair: degeneration of Science to Re-Science?	Environmental Sciences Europe (2015), Vol. 27, No. 19, (29 August 2015) p	Not relevant for the assessment of ED effects: Commentary of Seralini paper retraction.	The RMS agrees with the applicant's justification.

Toxicology	Faria M. A.	2015	Glyphosate, neurological diseases - and the scientific method	Surgical neurology international, (2015) Vol. 6, pp. 132	Not relevant for the assessment of ED effects: LETTER -> Comments on Samsel and Seneff (ref 2324, rated not relevant for RA)	The RMS agrees with the applicant's justification.
Toxicology	Feldman V.	2014	Neurodevelopmental toxicity: Still more questions than answers.	The Lancet Neurology, (July 2014) Vol. 13, No. 7 pp. 645-646	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Letter to Editor	The RMS agrees with the applicant's justification.
Toxicology	Fluegge K. et al.	2017	Exploring the potential confounder of nitrogen fertilizers in the relationship between pesticide exposures and risk of leukemia: a Poisson regression wit two-way fixed-effects analysis	Chinese Journal of Cancer, Vol. 36, 20170101	Not relevant for the assessment of ED effects: Letter to editor, focuses on nitrogen fertilizers.	The RMS agrees with the applicant's justification.
Toxicology	Fluegge K. et al.	2017	Exposure to ambient PM10 and nitrogen dioxide and ADHD risk: A reply to Min & Min (2017).	Environment International, (JUN 2017) Vol. 103, pp. 109-110	Not relevant for the assessment of ED effects: No new data.	The RMS agrees with the applicant's justification.
Toxicology	Fluegge K. et al.	2016	Glyphosate Use Predicts Healthcare Utilization for ADHD in the Healthcare Cost and Utilization Project net (HCUPnet): A Two-Way Fixed-Effects Analysis.	Polish Journal of Environmental Studies, (2016) Vol. 25, No. 4, pp. 1489-1503	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: No new information without clear relevance for the risk assessment.	The RMS agrees with the applicant's justification.
Toxicology	Fluegge K. R. et al.	2015	Glyphosate Use Predicts ADHD Hospital Discharges in the Healthcare Cost and Utilization Project Net (HCUPnet): A Two-Way Fixed-Effects Analysis.	PloS one, (2015) Vol. 10, No. 8, pp. e0133525	Not relevant for the assessment of ED effects: Retracted publication.	The RMS agrees with the applicant's justification.
Toxicology	Ford B. et al.	2017	Mapping Proteome-wide Targets of Glyphosate in Mice.	Cell chemical biology, (2017 Feb 16) Vol. 24, No. 2, pp. 133-140	Not relevant for the assessment of ED effects: This publication is considered not relevant because intraperitoneal injection was used which is an inappropriate route of administration for the occupational and food risk assessment of glyphosate.	The RMS agrees with the applicant's justification.

Toxicology	Fortes C. et al.	2016	Occupational Exposure to Pesticides With Occupational Sun Exposure Increases the Risk for Cutaneous Melanoma	Journal of occupational and environmental medicine, (2016 Apr) Vol. 58, No. 4, pp. 370-5	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: No specific analyses for glyphosate. Interviewers were not impartial. Recall bias may produce spurious positive associations.	The RMS agrees with the applicant's justification.
Toxicology	Freddo N. et al	2019	Isoflavone quantitation in soymilk: Genistein content and its biological effect	CYTA – JOURNAL OF FOOD 2019, VOL. 17, NO. 20-24	Not relevant for the assessment of ED effects: This publication is not relevant for the risk assessment of glyphosate in relation to ED as it mainly concerns the development of a bioanalytical method for the analysis of genistein and glyphosate in soymilk. The biological endpoint selected (anxiety) and the test system used (elevated plus maze test) are not suitable for regulatory use.	The RMS agrees with the applicant's justification.
Toxicology	Gallegos C. E. et al.	2016	Exposure to a glyphosate-based herbicide during pregnancy and lactation induces neurobehavioral alterations in rat offspring.	Neurotoxicology, (2016 Mar) Vol. 53, pp. 20-28	Not relevant for the assessment of ED effects: Formulation tested in vivo via drinking water (Glifloglex, 48% glyphosate, Gleba S.R.L., Argentina)	The RMS agrees with the applicant's justification.
Toxicology	De Castilhos Ghisi N. et al.	2016	Does exposure to glyphosate lead to an increase in the micronuclei frequency? A systematic and meta- analytic review.	Chemosphere, (2016 Feb) Vol. 145, pp. 42-54	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: No new data presented, only a compilation of pooled glyphosate and formulated product meta-anslysis is provided.	The RMS agrees with the applicant's justification.
Toxicology	Goldstein D. A et al.	2014	Neurodevelopmental toxicity: Still more questions than answers.	The Lancet Neurology, (July 2014) Vol. 13, No. 7 pp. 645	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Letter to Editor	The RMS agrees with the applicant's justification.
Toxicology	Good P.	2018	Evidence the U.S. autism epidemic initiated by acetaminophen (Tylenol) is aggravated by oral antibiotic amoxicillin/clavulanate (Augmentin) and now exponentially by herbicide glyphosate (Roundup).	Clinical nutrition ESPEN, (20180200) Vol. 23, pp 171-183	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case c) Relevance cannot be determined: Potential effects on gut microbes are not part of the risk assessments and suitable scientific approaches to assess these effects are not specified, thus relevance of these effects remained unclear. This paper contains no new data. It uses	The RMS agrees with the applicant's justification. Considering the non-ED endpoints, RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.

					computer algor=ithms to make associations that are not proved. It claims that glyphosate impacts methionine and tryptophan and ignores that these amino acids are essential for human diet as microbially derived amino acids are only available via coprophagy.	
Toxicology	Gress S. et al.	2016	Dig1 protects against locomotor and biochemical dysfunctions provoked b Roundup.	BMC complementary and alternative medicine, (2016 Jul 22) Vol. 16, pp. 234	Not relevant for the assessment of ED effects: A glyphosate based herbicide was administered to rats in drinking water.	The RMS agrees with the applicant's justification.
Toxicology	Grunewald W. et al.	2013	Comment on "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize" by Seralini et al.	Food and Chemical Toxicology, (2013) Vol. 53, pp. 447-448	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Letter to Editor	The RMS agrees with the applicant's justification.
Toxicology	Guerrero S. M. et al.	2017	Neonatal exposure to a glyphosate based herbicide alters the developmen of the rat uterus.	Toxicology, (2017 Feb 01) Vol. 376, pp. 2-14	Not relevant for the assessment of ED effects: Formulation tested in vivo via sub-cutaneous injection (Roundup FULL II, 66 2% potassium salt).	The RMS agrees with the applicant's justification.
Toxicology	Guilherme S. e al.	2014	Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure?.	Aquatic toxicology (Amsterdam, Netherlands), (2014 Oct) Vol. 155, pp. 213-21	Not relevant for the assessment of ED effects: A glyphosate based herbicide was tested in aquatic species.	The RMS agrees with the applicant's justification.
Toxicology	Haggard D. et al.	2018	High-throughput H295R steroidogenesis assay: utility as an alternative and a statistical approach t characterize effects on steroidogenesi	Toxicological Sciences, (2018) Vol. 162, No. 2, pp. 509-534	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate in terms of ED as it concerns the validation of the high-throughput H295R steroidogenesis assay.	The RMS agrees with the applicant's justification.
Toxicology	Halwachs S. et al.	2016	Assessment of ABCG2-mediated transport of pesticides across the rabbit placenta barrier using a novel MDCKII in vitro model.	Toxicology and applied pharmacology, (20160815) Vol. 305, pp. 66-74	Not relevant for the assessment of ED effects: No adverse effects, no relevance to human health risk assessment.	The RMS agrees with the applicant's justification.

Toxicology	Hamdaoui L. e al.	2016	Nephrotoxicity of Kalach 360 SL: biochemical and histopathological findings.	Toxicology mechanisms and methods, (2016 Nov) Vol. 26, No. 9, pp. 685-691	Not relevant for the assessment of ED effects: Formulation tested (Kalach 360 SL) in vivo.	The RMS agrees with the applicant's justification.
Toxicology	Hammond B. e al.	2013	A Comment on "Seralini, G.-E., et al., Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chemical Toxicol. (2012)," http://dx.doLorg/10.1016/j.fct.2012.0 .005.	Food and Chemical Toxicology, (MAR 2013) Vol 53, pp. 444-449	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Letter to Editor	The RMS agrees with the applicant's justification.
Toxicology	Han J. et al.	2016	Determination of glyphosate and its metabolite in emergency room in Korea.	Forensic science international, (2016 Aug) Vol. 265, pp. 41-6	Not relevant for the assessment of ED effects: Analytical method development in human blood	The RMS agrees with the applicant's justification.
Toxicology	Haskovi E. et al.	2016	Effects of Glyphosate on Enzyme Activity and Serum Glucose in Rats Rattus norvegicus	Acta veterinaria (2016), Vol. 66, No. 2, pp. 214- 221	Not relevant for the assessment of ED effects: Only liver enzymes measured after 15 days dermal application of formulated product (Total 480 SL, Croatia)	The RMS agrees with the applicant's justification.
Toxicology	Heritier L. et al.	2017	Oxidative stress induced by glyphosate-based herbicide on freshwater turtles.	Environmental toxicology and chemistry, (20171200) Vol. 36, No. 12, pp. 3343-3350	Not relevant for the assessment of ED effects: GBH tested on turtles.	The RMS agrees with the applicant's justification.
Toxicology	Hong Y. et al.	2017	Effects of glyphosate on immune responses and haemocyte DNA damage of Chinese mitten crab, Eriocheir sinensis.	Fish & shellfish immunology, (2017 Dec) Vol. 71, pp. 19-27	Not relevant for the assessment of ED effects: high doses of GBH to crabs	The RMS agrees with the applicant's justification.
Toxicology	Hoppin J. A. e al.	2017	Pesticides are Associated with Allergic and Non-Allergic Wheeze among Male Farmers.	Environmental health perspectives, (20170400) Vol. 125, No. 4, pp. 535-543	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: The exposure and outcome data were concurrent, so a temporal relationship could not	The RMS agrees with the applicant's justification.

					be established. The extraordinary number of positive statistically significant findings mitigates against interpreting any one finding as likely to be causal.	
Toxicology	Hua J. et al.	2018	Differential microRNA expression in the prefrontal cortex of mouse offspring induced by glyphosate exposure during pregnancy and lactation.	Experimental and therapeutic medicine, (2018) Vol. 15, No. 3, pp. 2457-2467	Not relevant for the assessment of ED effects: Formulation is not relevant for the ED risk assessment	The RMS agrees with the applicant's justification.
Toxicology	Ilyushina N. et al.	2019	Maximum tolerated doses and erythropoiesis effects in the mouse bone marrow by 79 pesticides' technical materials assessed with the micronucleus assay.	Toxicology Reports, (2019) Vol. 6, pp. 105-110	Not relevant for the assessment of ED effects: Releated to genotoxicity, not relevant to ED.	The RMS agrees with the applicant's justification.
Toxicology	Indirakshi J. et al.	2017	Toxic Epidermal Necrolysis and Acut Kidney Injury due to Glyphosate Ingestion.	Indian journal of critical care medicine : peer- reviewed, official publication of Indian Society of Critical Care Medicine, (2017 Mar) Vol. 21, No. 3 pp. 167-169	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Glyphosate based formulations are not known to cause TEN which is a t-cell mediated type IV hypersensitivity reaction. >1% of glyphosate is absorbed through the skin and large ingestions have caustic effects on th GI tract which can result in multiorgan failure.	The RMS agrees with the applicant's justification.
Toxicology	Ingaramo P. I. et al.	2017	Neonatal exposure to a glyphosate-based herbicide alters uterine decidualization in rats.	Reproductive toxicology (Elmsford, N.Y.), (2017 Oct) Vol. 73, pp. 87-95	Not relevant for the assessment of ED effects: Formulation tested in vivo via subcutaneous injection (undisclosed brand, 66.2% potassium salt; 54% glyphosate acid).	The RMS agrees with the applicant's justification.
Toxicology	Ingaramo P. I. et al.	2016	Effects of neonatal exposure to a glyphosate-based herbicide on female rat reproduction.	Reproduction (Cambridge, England), (20161100) Vol. 152, No. 5, pp. 403-15	Not relevant for the assessment of ED effects: Formulation tested in vivo (66.2%, potassium salt)	The RMS agrees with the applicant's justification.

Toxicology	Iummato M. M. et al.	2017	Effect of glyphosate acid on biochemical markers of periphyton exposed in outdoor mesocosms in the presence and absence of the mussel Limnoperna fortunei.	Environmental toxicology and chemistry, (20170700) Vol. 36, No. 7, pp. 1775-1784	Not relevant for the assessment of ED effects: endpoints not relevant to human health.	The RMS agrees with the applicant's justification.
Toxicology	Iwai K. et al.	2014	Utility of upper gastrointestinal endoscopy for management of patient with roundup poisoning.	Journal of Clinical Toxicology (2014), Vol. 4, No. 6, 1000218 p	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article discusses the use of endoscopy to treat formulated glyphosate overdose and medical management of suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
Toxicology	Jayasumana C. et al.	2014	Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka?.	International journal of environmental research an public health, (2014 Feb 20) Vol. 11, No. 2, pp. 2125-47	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Presents a hypothesis which is not tested, only discussed.	The RMS agrees with the applicant's justification.
Toxicology	Jayasumana C. et al.	2015	Simultaneous exposure to multiple heavy metals and glyphosate may contribute to Sri Lankan agricultural nephropathy.	BMC nephrology, (2015 Jul 11) Vol. 16, pp. 103	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Presents a hypothesis which is not tested, only discussed	The RMS agrees with the applicant's justification.
Toxicology	Jomichen J. et al.	2017	Australian work exposures studies: occupational exposure to pesticides.	Occupational and environmental medicine, (20170100) Vol. 74, No. 1, pp. 46-51	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Occupational exposure survey.	The RMS agrees with the applicant's justification.
Toxicology	Jovic-Stosic J. et al.	2016	Intravenous lipid emulsion in treatment of cardiocirculatory disturbances caused by glyphosate- surfactant herbicide poisoning.	Vojnosanitetski pregled, (2016 Apr) Vol. 73, No. 4, pp. 390-2	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Medical case of intentional ingestion.	The RMS agrees with the applicant's justification.

Toxicology	Kasuba V. et al.	2017	Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line	Environmental science and pollution research international, (2017 Aug) Vol. 24, No. 23, pp. 19267-19281	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	The RMS agrees with the applicant's justification.
Toxicology	Kawagashira Y. et al.	2017	Vasculitic Neuropathy Following Exposure to a Glyphosate-based Herbicide.	Internal medicine (Tokyo, Japan), (2017) Vol. 56, No. 11, pp. 1431-1434	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article discussed the development of painful discoloration of the toes and feet 4 months after the patient sprayed formulated glyposate to crops. Interestingly enough, the patient was taking warfarin therapeutically, which can cause the well-described "purple toe syndrome". There is not a mechanism by which sprayed formulated glyphosate can be absorbed by the skin and directly impact small vasculature or neurons in the feet.	The RMS agrees with the applicant's justification.
Toxicology	Kim E. et al.	2016	Patterns of drugs & poisons in southern area of South Korea in 2014.	Forensic Science International, (1 Dec 2016) Vol. 269, pp. 50-55	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This is an article describing the chemicals/pharmaceuticals that were used in fatal overdoses that were forensically evaluated at the Busan Institute of National Forensic Services. Out of 606 fatalities, agricultural chemicals were involved in 5 and glyphosate was detected in 2 of the cases.	The RMS agrees with the applicant's justification.
Toxicology	Kim Y. H. et al.	2014	Heart rate-corrected QT interval predicts mortality in glyphosate-surfactant herbicide-poisoned patients	The American journal of emergency medicine, (2014 Mar) Vol. 32, No. 3, pp. 203-7	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article discusses the utility of the QTc interval to predict mortality in suicidal ingestions of glyphosate-based formulations. It is not unexpected for critically ill patients to develop a long QTc.	The RMS agrees with the applicant's justification.

Toxicology	Kim Y. H. et al.	2016	Prognostic Factors in Emergency Department Patients with Glyphosate Surfactant Intoxication: Point-of-Care Lactate Testing.	Basic & clinical pharmacology & toxicology, (2016 Dec) Vol. 119, No. 6, pp. 604-610	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This study evaluated the use of lactate as a predictor of mortality and found a statistically significant association between a serum lactate of 4.7mmol/L and mortality in formulated glyphosate overdoses. This is not surprising as caustic injury due to detergent- like surfactants will cause cell death and thereby increase lactate levels. This article discusses predictors of mortality in suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
Toxicology	Knudsen L. E. et al.	2017	Biomonitoring of Danish school children and mothers including biomarkers of PBDE and glyphosate.	Reviews on environmental health, (2017 Sep 26) Vol. 32, No. 3, pp. 279-290	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: All glyphosate levels many orders of magnitude lower than the ADI.	The RMS agrees with the applicant's justification.
Toxicology	Kongtip P. et al.	2017	Glyphosate and Paraquat in Maternal and Fetal Serums in Thai Women.	Journal of agromedicine, (2017) Vol. 22, No. 3, pp. 282-289	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	The RMS agrees with the applicant's justification.
Toxicology	Kubsad D. et al.	2019	Assessment of Glyphosate Induced Epigenetic Transgenerational Inheritance of Pathologies and Sperm Epimutations: Generational Toxicology	Scientific Reports, (2019) Vol. 9, No. 1, pp. 1-17	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate with relation to ED because of the use of an inappropriate route of administration (intraperitoneal injection).	The RMS agrees with the applicant's justification.
Toxicology	Kumar S. et al.	2014	Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation	Toxicology, (2014 Nov 05) Vol. 325, pp. 42-51	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	The RMS agrees with the applicant's justification.

Toxicology	Kurenbach B. et al.	2017	Herbicide ingredients change Salmonella enterica sv. Typhimurium and Escherichia coli antibiotic responses.	Microbiology (Reading, England), (2017 Nov 17)	Not relevant for the assessment of ED effects: high doses to an in vitro system.	The RMS agrees with the applicant's justification.
Toxicology	Kurenbach B. et al.	2015	Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium.	mBio, (2015 Mar 24) Vol. 6, No. 2	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Endpoints at doses tested not relevant to residue levels or to human health.	The RMS agrees with the applicant's justification.
Toxicology	Kwiatkowska M. et al.	2014	The effect of metabolites and impurities of glyphosate on human erythrocytes (in vitro).	Pesticide biochemistry and physiology, (2014 Feb Vol. 109, pp. 34-43	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because the in vitro concentrations used are in the mM range and the impurities were tested at the same concentrations as glyphosate which will never occur in practice.	The RMS agrees with the applicant's justification.
Toxicology	Kwiatkowska M. et al.	2016	The Impact of Glyphosate, Its Metabolites and Impurities on Viability, ATP Level and Morphological changes in Human Peripheral Blood Mononuclear Cells.	PloS one, (2016) Vol. 11, No. 6, pp. e0156946	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because the in vitro concentrations used are in the mM range and the impurities were tested at the same concentrations as glyphosate which will never occur in practice.	The RMS agrees with the applicant's justification.
Toxicology	Landrigan P.	2018	Pesticides and Human Reproduction.	JAMA Internal Medicine, (2018) Vol. 178, No. 1, pp. 26-27	Not relevant for the assessment of ED effects: Secondary source of information not related to ED	The RMS agrees with the applicant's justification.
Toxicology	Larsen K. E. et al.	2016	The herbicide glyphosate is a weak inhibitor of acetylcholinesterase in rats.	Environmental toxicology and pharmacology, (2016 Jul) Vol. 45, pp. 41-4	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because the concentrations used for in vitro testing were all in the mM range.	The RMS agrees with the applicant's justification.

Toxicology	Larsen K. et al	2014	Effects of Sublethal Exposure to a Glyphosate-Based Herbicide Formulation on Metabolic Activities of Different Xenobiotic-Metabolizing Enzymes in Rats.	International journal of toxicology, (20140700) Vol. 33, No. 4, pp. 307-318	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4 1 case b) Relevant but supplementary information: Formulation tested in vivo via drinking water (Roundup FULL II, 662 g/L potassium salt).	The RMS agrees with the applicant's justification.
Toxicology	Le Tien D. et al.	2013	Comments on "Long term toxicity of Roundup herbicide and a Roundup-tolerant genetically modified maize"	Food and Chemical Toxicology, (2013) Vol. 53, pp. 443-444	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Letter to Editor	The RMS agrees with the applicant's justification.
Toxicology	Lee D. H. et al	2017	Severe glyphosate-surfactant intoxication: Successful treatment with continuous renal replacement therapy.	Hong Kong Journal of Emergency Medicine, (January 2017) Vol. 24, No. 1, pp. 40-44	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This is a report about multiorgan failure and the use of dialysis after suicidal ingestion of formulated glyphosate and should not impact re-registration.	The RMS agrees with the applicant's justification.
Toxicology	Lemma T. et a	2019	Combined effect of glyphosphate, saccharin and sodium benzoate on rat	Biophysical chemistry, (2019 Jul) Vol. 250, pp. 106176	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Novel assays and endpoints not applicable/reliable for risk assessment.	The RMS agrees with the applicant's justification.
Toxicology	Leveroni F. A. et al.	2017	Genotoxic response of blood, gill and liver cells of Piaractus mesopotamicu after an acute exposure to a glyphosate-based herbicide	Caryologia (2017), Vol. 70, No. 1, pp. 21-28	Not relevant for the assessment of ED effects: Formulation tested in aquatic species (Roundup Full II; 66.2% glyphosate potassium salt; CAS no. 70901-12-1)	The RMS agrees with the applicant's justification.
Toxicology	Lewis M. M. e al.	2017	Lateralized basal ganglia vulnerability to pesticide exposure in asymptomatic agricultural workers	Toxicological Sciences, (2017) Vol. 159, No. 1, pp. 170-178	Not relevant for the assessment of ED effects: Results not correlated to exposure to glyphosate	The RMS agrees with the applicant's justification.

Toxicology	Li M. et al.	2016	Multi-tissue metabolic responses of goldfish (Carassius auratus) exposed to glyphosate-based herbicide.	Toxicology Research, (2016) Vol. 5, No. 4, pp. 1039-1052	Not relevant for the assessment of ED effects: glyphosate based herbicide tested on goldfish. Endpoints not relevant to human health risk assessment	The RMS agrees with the applicant's justification.
Toxicology	Lieshchova M. A. et al.	2018	Combined effect of glyphosphate, saccharin and sodium benzoate on rats.	Regulatory Mechanisms in Biosystems (2018), Vol. 9, No. 4, pp. 591-597, many ref	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4 1 case b) Relevant but supplementary information: Substantially lower water consumption in glyphosate only group confounds data and makes endpoint comparisons meaningless.	The RMS agrees with the applicant's justification.
Toxicology	Loomba R. S.	2016	Prevalence of isomerism from a European registry: Live births, fetal deaths, and terminations of pregnancy	Congenital Anomalies, (NOV 2016) Vol. 56, No. 6, pp. 256-257	Not relevant for the assessment of ED effects: No mention of glyphosate or AMPA.	The RMS agrees with the applicant's justification.
Toxicology	Lopez G. E. C. et al.	2017	Micronuclei and other nuclear abnormalities on Caiman latirostris (Broad-snouted caiman) hatchlings after embryonic exposure to different pesticide formulations.	Ecotoxicology and environmental safety, (2017 Feb) Vol. 136, pp. 84-91	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This study looks at the impact of pesticide formulations on the nuclear developments of Caimen embryos via topical application to their eggs shells after laying. The endpoints achieved cannot be related to EU risk assessment.	The RMS agrees with the applicant's justification.
Toxicology	Luaces J. P. et al.	2017	Genotoxic effects of Roundup Full II® on lymphocytes of Chaetophractu villosus (Xenarthra, Mammalia): In vitro studies.	PloS one, (2017) Vol. 12, No. 8, pp. e0182911	Not relevant for the assessment of ED effects: Formulation tested in vivo (Roundup Full II, 66.2% glyphosate, Argentina)	The RMS agrees with the applicant's justification.
Toxicology	Luna S and Rosso S.	2019	Glyphosate exposure impairs neurona connectivity and spatial learning in rats.	ASN Neuro, (2019) Vol. 11, pp. 52-53. Abstract Number: P65. Meeting Info: 33rd Congress of the Argentine Society for Research in Neuroscience. Cordoba, Argentina. 24 Oct 2018-26 Oct 2018	Not relevant for the assessment of ED effects: related to neurotoxicity, not ED	The RMS agrees with the applicant's justification.

Toxicology	Luo L. et al.	2017	In vitro cytotoxicity assessment of roundup (glyphosate) in L-02 hepatocytes.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes, (2017 Jun 03) Vol. 52, No. 6, pp. 410-417	Not relevant for the assessment of ED effects: Formulation tested in vitro (Roundup, 41% isopropylamine salt; Belgium). Effects due to high dosing of surfactant in vitro.	The RMS agrees with the applicant's justification.
Toxicology	Malagoli C. et al.	2016	Passive exposure to agricultural pesticides and risk of childhood leukemia in an Italian community.	International journal of hygiene and environmenta health, (20161100) Vol. 219, No. 8, pp. 742-748	Not relevant for the assessment of ED effects: No specific analyses for glyphosate. Very small case control study with a very speculative exposure variable	The RMS agrees with the applicant's justification.
Toxicology	Mao Q. et al.	2018	The Ramazzini Institute 13-week pilo study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome	Environmental Health , Vol. 17, (2018) pp. 12940	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate in relation to ED because no ED related endpoint was investigated (gut microbiome in dams and offspring).	The RMS agrees with the applicant's justification. Considering the non-ED endpoints, RMS requested a study summary in order to further justify the categorization. Refer to B.6.8.2 for an assessment of the study.
Toxicology	Mao Y. et al.	2015	Effect of glyphosate on serum biochemical indices of exposed workers	Zhongguo Gongye Yixue Zazhi, (2015) Vol. 28, No. 5, pp. 362-364	Not relevant for the assessment of ED effects: The worker protections and manufacturing processes in China do not likely reflect Western occupational exposure scenarios.	The RMS agrees with the applicant's justification.
Toxicology	Marcoccia D. et al.	2017	Food components and contaminants a (anti)androgenic molecules.	Genes and Nutrition, (16 Feb 2017) Vol. 12, No. 1 arn. 6	Not relevant for the assessment of ED effects: Discusses some glyphosate literature, but no new data	The RMS agrees with the applicant's justification.
Toxicology	Martens M. et al.	2019	Toxicology and human health risk assessment of polyethoxylated tallow amine surfactant used in glyphosate formulations.	Regulatory toxicology and pharmacology (2019) Vol. 107, pp. 104347	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate in relation to ED as the surfactant used in glyphosate formulations was tested and evaluated instead of glyphosate.	The RMS agrees with the applicant's justification.

Toxicology	Martini C. N. e al.	2016	Glyphosate Inhibits PPAR Gamma Induction and Differentiation of Preadipocytes and is able to Induce Oxidative Stress.	Journal of biochemical and molecular toxicology, (2016 Aug) Vol. 30, No. 8, pp. 404-13	Not relevant for the assessment of ED effects: Formulation tested in vitro at a single high dose in the mM range (Glifosato Atanor, 48% isopropylamine salt, 35.6% glyphosate, Argentina)	The RMS agrees with the applicant's justification.
Toxicology	Mesnage R. et al.	2015	Transcriptome profile analysis reflect rat liver and kidney damage following chronic ultra-low dose Roundup exposure.	Environmental health : a global access science source, (2015 Aug 25) Vol. 14, pp. 70	Not relevant for the assessment of ED effects: Formulation tested (Grand Travaux Plus (450 g/L, Belgium) for non-validated endpoints.	The RMS agrees with the applicant's justification.
Toxicology	Mesnage R. et al.	2015	Potential toxic effects of glyphosate and its commercial formulations below regulatory limits.	Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, (2015 Oct) Vol. 84, pp. 133-53	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: review, secondary source.	The RMS agrees with the applicant's justification.
Toxicology	Mesnage R. et al.	2017	Facts and Fallacies in the Debate on Glyphosate Toxicity.	Frontiers in public health, (2017) Vol. 5, pp. 316	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: review, secondary source	The RMS agrees with the applicant's justification.
Toxicology	Mesnage R. et al.	2017	Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide.	Scientific reports, (20170109) Vol. 7, pp. 39328	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Formulation tested (Roundup, composition not described). Livers obtained from research of republished reteated Seralini rat study.	The RMS agrees with the applicant's justification.
Toxicology	Mesnage R. et al.	2014	Major pesticides are more toxic to human cells than their declared active principles.	BioMed research international, (2014) Vol. 2014, pp. 179691	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: In vitro cytotoxicity data at high doses not informative for hazard characterization.	The RMS agrees with the applicant's justification.
Toxicology	Mesnage R. et al.	2018	Ignoring Adjuvant Toxicity Falsifies the Safety Profile of Commercial Pesticides	FRONTIERS IN PUBLIC HEALTH, (22 JAN 2018) Vol. 5	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	The RMS agrees with the applicant's justification.

Toxicology	Mills P. J. et al	2017	Excretion of the Herbicide Glyphosat in Older Adults Between 1993 and 2016.	JAMA, (20171024) Vol. 318, No. 16, pp. 1610-1611	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Not relevant for EU toxicology risk assessment but supplementary information on human exposure.	The RMS agrees with the applicant's justification.
Toxicology	Mohamed F. e al.	2016	Mechanism-specific injury biomarker predict nephrotoxicity early following glyphosate surfactant herbicide (GPSH) poisoning.	Toxicology letters, (2016 Sep 06) Vol. 258, pp. 1-10	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article discusses the use of biomarkers to predict kidney damage in formulated glyphosate overdose and predictors of nephrotoxicity in suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.
Toxicology	Moon J. M. et al.	2016	The characteristics of emergency department presentations related to acute herbicide or insecticide poisoning in South Korea between 2011 and 2014.	Journal of toxicology and environmental health. Part A, (2016) Vol. 79, No. 11, pp. 466-76	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This study showed a decrease in the case fatality rate of suicidal pesticide ingestions between 2011-2014 in South Korea. This clearly demonstrates that herbicides with a lower acute toxicity profile are associated with lower mortality in suicidal ingestions.	The RMS agrees with the applicant's justification.
Toxicology	Nakae H. et al.	2015	Paralytic ileus induced by glyphosate intoxication successfully treated using Kampo medicine.	Acute medicine & surgery, (20150700) Vol. 2, No 3, pp. 214-218	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article describes alternative medicine therapies that were used to treat a Japanese woman with a paralytic ileus after glyphosate ingestion. It is not uncommon for patients in a critical care setting to develop an ileus. These tend to resolve on their own without intervention. It cannot be commented on whether this intervention increases GI motility. This should not impact re-registration.	The RMS agrees with the applicant's justification.

Toxicology	Nardi J. et al.	2017	Prepubertal subchronic exposure to soy milk and glyphosate leads to endocrine disruption.	Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, (2017 Feb) Vol. 100, pp. 247-252	Not relevant for the assessment of ED effects: Formulation co-dosed with phytoestrogen containing vehicle.	The RMS agrees with the applicant's justification.
Toxicology	Naz S. et al.	2019	Effect of glyphosate on hematological and biochemical parameters of Rabbit (Oryctolagus cuniculus)	Pure and Applied Biology (2019), Vol. 8, No. 1, pp. 78-92	Not relevant for the assessment of ED effects: Not relevant: Tested formulation 48%IPA (36.6% a.e.) . Only hematology and clinical chemistry values reported.	The RMS agrees with the applicant's justification.
Toxicology	Nielsen L. N. c r. et al.	2017	Glyphosate has limited short-term effects on commensal bacterial community composition in the gut environment due to sufficient aromati amino acid levels	Environmental pollution (2017)	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case c) Relevance cannot be determined: The investigation of potential effects on the gut microbiome of ruminants is not a data requirement for the approval of pesticides and suitable test protocols to assess these effects are not specified in the form of official guidance documents. Therefore, the relevance of the publication is unclear.	The RMS agrees with the applicant's justification.
Toxicology	Owagboriaye F. O. et al.	2017	Reproductive toxicity of Roundup herbicide exposure in male albino rat.	Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie, (2017 Sep 05) Vol. 69, No. 7, pp. 461-468	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Formulation tested in vivo (Roundup 441 g/L potassium salt, 360 g/L a.e.)	The RMS agrees with the applicant's justification. Refer to B.6.6.3.1 for an assessment of the study in the context of CA 5.6.
Toxicology	Ozaki T. et al.	2017	Severe Glyphosate-Surfactant Intoxication Successfully Treated With Continuous Hemodiafiltration and Direct Hemoperfusion: Case Report.	Therapeutic apheresis and dialysis : official peer- reviewed journal of the International Society for Apheresis, the Japanese Society for Apheresis, the Japanese Society for Dialysis Therapy, (20170600 Vol. 21, No. 3, pp. 296-297	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article discusses the use of haemodialysis and haemofiltration in formulated glyphosate overdoses.This article discusses medical management of suicidal ingestions and therefore should not impact registration decisions.	The RMS agrees with the applicant's justification.

Toxicology	Parajuli K. R. et al.	2015	Aminomethylphosphonic acid and methoxyacetic acid induce apoptosis in prostate cancer cells.	International journal of molecular sciences, (2015 May 22) Vol. 16, No. 5, pp. 11750-65	Not relevant for the assessment of ED effects: Therapeutic use of AMPA evaluated.	The RMS agrees with the applicant's justification.
Toxicology	Park S. et al.	2016	Concurrent Hemoperfusion and Hemodialysis in Patients with Acute Pesticide Intoxication.	Blood Purification, (1 Dec 2016) Vol. 42, No. 4, pp. 329-336	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article describes the use of haemodialysis and haemoperfusion in pesticide overdoses. Out of 383 pesticide ingestions 110 were glyphosate formulations. Of the 80 deaths reported 12 of them were glyphosate. This article describes a possibly beneficial modality of treating severe pesticide overdose and should not impact re-registration.	The RMS agrees with the applicant's justification.
Toxicology	Parks C. G. et al.	2016	Rheumatoid Arthritis in Agricultural Health Study Spouses: Associations with Pesticides and Other Farm Exposures.	Environmental health perspectives, (2016 Nov) Vol. 124, No. 11, pp. 1728-1734	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Lack of information about glyphosate frequency of use and timing of use.	The RMS agrees with the applicant's justification.
Toxicology	Perego M. C. e al.	2017	Influence of a Roundup formulation on glyphosate effects on steroidogenesis and proliferation of bovine granulosa cells in vitro.	Chemosphere, (2017 Dec) Vol. 188, pp. 274-279	Not relevant for the assessment of ED effects: In vitro formulation effects only, not glyphosate alone.	Publication included in the dossier. Please refer to Volume 3 CA B.6.6 for an assessment.
Toxicology	Perez-Torres I. et al.	2017	Beneficial Effects of the Amino Acid Glycine.	Mini reviews in medicinal chemistry, (2017) Vol. 17, No. 1, pp. 15-32	Not relevant for the assessment of ED effects: No glyphosate data	The RMS agrees with the applicant's justification.
Toxicology	Picetti E. et al.	2017	Glyphosate ingestion causing multiple organ failure: A near-fatal case report	Acta Biomedica, (2017) Vol. 88, No. 4, pp. 533-536	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This is a report concerning multiorgan failure following suicidal ingestion of formulated glyphosate and should not impact re-	The RMS agrees with the applicant's justification.

					registration.	
Toxicology	Portier C. J. et al.	2016	Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA).	Journal of epidemiology and community health, (20160800) Vol. 70, No. 8, pp. 741-5	Not relevant for the assessment of ED effects: This publication is considered not relevant because it is not based on experimental data.	The RMS agrees with the applicant's justification.
Toxicology	Portier C. J. et al.	2017	Re: Tarazona et al. (2017): Glyphosat toxicity and carcinogenicity: a review of the scientific basis of the European Union assessment and its differences with IARC. doi: 10.1007/s00204-017-1962-5.	Archives of toxicology, (20170900) Vol. 91, No. 9, pp. 3195-3197	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: letter to editor, ref to Tarazona_2017	The RMS agrees with the applicant's justification.
Toxicology	Pouokam G. B et al.	2017	A Pilot Study in Cameroon to Understand Safe Uses of Pesticides in Agriculture, Risk Factors for Farmers' Exposure and Management of Accidental Cases.	Toxics, (2017 Nov 01) Vol. 5, No. 4	Not relevant for the assessment of ED effects: No informative data on glyphosate	The RMS agrees with the applicant's justification.
Toxicology	Ramsden J. J.	2017	Assaults on health.	Journal of Biological Physics and Chemistry, (2017) Vol. 17, No. 1, pp. 3-7	Not relevant for the assessment of ED effects: Commentary on various threats to human health.	The RMS agrees with the applicant's justification.
Toxicology	Rebai O. et al.	2017	Morus alba leaf extract mediates neuroprotection against glyphosate-induced toxicity and biochemical alterations in the brain.	Environmental science and pollution research international, (2017 Apr) Vol. 24, No. 10, pp. 9605-9613	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Formulation administered via i.p. injection (described as a commercial formulation registered in the Tunisian Ministry of Agriculture).	The RMS agrees with the applicant's justification.

Toxicology	Ren X. et al.	2019	Effects of chronic glyphosate exposure to pregnant mice on hepatic lipid metabolism in offspring.	Environmental pollution, (2019) Vol. 254, pp. 112906	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate in the context of ED as effects were investigated on hepatic lipid metabolism in offspring which are not considered in an EATS assessment.	The RMS agrees with the applicant's justification. Refer to B.6.6.3.1 for an assessment of the study.
Toxicology	Rendon-von O J. et al.	2017	Glyphosate Residues in Groundwater, Drinking Water and Urine of Subsistence Farmers from Intensive Agriculture Localities: A Survey in Hopelchen, Campeche, Mexico.	International journal of environmental research an public health, (20170603) Vol. 14, No. 6	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: No new information without clear relevance for the risk assessment	The RMS agrees with the applicant's justification.
Toxicology	Roongruangch i J. et al.	2018	The teratogenic effects of glyphosate based herbicide (GBH) on the development of chick embryos.	Siriraj Medical Journal (2018), Volume 70, Number 5, pp. 419-428	Not relevant for the assessment of ED effects: Test substance was a glyphosate based formulation not relevant for the risk assessment	The RMS agrees with the applicant's justification.
Toxicology	Roustan A. et al.	2014	Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation product before and after photoactivation	Chemosphere, (2014 Aug) Vol. 108, pp. 93-100	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	The RMS agrees with the applicant's justification.
Toxicology	Samsel A. et al	2013	Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance.	Interdisciplinary toxicology, (2013 Dec) Vol. 6, No. 4, pp. 159-84	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
Toxicology	Samsel A. et al	2015	Glyphosate, pathways to modern diseases III: Manganese, neurological diseases, and associated pathologies.	Surgical neurology international, (2015) Vol. 6, pp. 45	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
Toxicology	Samsel A. et al	2015	Glyphosate, pathways to modern diseases IV: cancer and related pathologies.	Journal of Biological Physics and Chemistry, (2015) Vol. 15, No. 3, pp. 121-159	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.

Toxicology	Samsel A. et al	2016	Glyphosate pathways to modern diseases V: Amino acid analogue of glycine in diverse proteins.	Journal of Biological Physics and Chemistry, (2016) Vol. 16, No. 1, pp. 9-46	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because it is	The RMS agrees with the applicant's justification.
					not based on experimental work and no epidemiologic methodology was followed.	
Toxicology	Samsel A. et al	2017	Glyphosate pathways to modern diseases VI: Prions, amyloidoses and autoimmune neurological diseases.	Journal of Biological Physics and Chemistry, (2017) Vol. 17, No. 1, pp. 8-32	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because it is not based on experimental work and no epidemiologic methodology was followed.	The RMS agrees with the applicant's justification.
Toxicology	Schaumburg L G. et al.	2016	Genotoxicity induced by Roundup® (Glyphosate) in tegu lizard (Salvator merianae) embryos.	Pesticide biochemistry and physiology, (2016 Jun) Vol. 130, pp. 71-78	Not relevant for the assessment of ED effects: A glyphosate based herbicide tested in lizard eggs.	The RMS agrees with the applicant's justification.
Toxicology	Schinasi L. et al.	2014	Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis.	International journal of environmental research an public health, (2014 Apr 23) Vol. 11, No. 4, pp. 4449-527	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case c) Relevance cannot be determined: The relevance of this paper is considered uncertain because it concerns a meta-analysis where the results were taken from available studies at face value. The authors had no way to correct for recall bias, confounding, etc. Therefore, the meta-RRs are in error to the extent that the studies included in the meta-analysis are in error.	AGG requested a study summary in order to further justify the categorization. Refer to B.6.5 for an assessment of the study.
Toxicology	Seneff S. et al.	2015	Death as a drug side effect in FAERS is glyphosate contamination a factor?	Agricultural Sciences (2015), Vol. 6, No. 12, pp. 1472-1501	Not relevant for the assessment of ED effects: Hypothesis discussed without any empirical data	The RMS agrees with the applicant's justification.

Toxicology	Seneff S. et al.	2017	Can glyphosate's disruption of the gut microbiome and induction of sulfate deficiency explain the epidemic in gout and associated diseases in the industrialized world?.	Journal of Biological Physics and Chemistry, (2017) Vol. 17, No. 2, pp. 53-76	Not relevant for the assessment of ED effects: Not relevant as the study is not based upon experimental work and no epidemiologic methodology was followed. Results and proposed mode of actions are pure speculation without any experimental proof.	The RMS agrees with the applicant's justification.
Toxicology	Seralini G. et al.	2014	Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modifie maize.	Environmental sciences Europe, (2014) Vol. 26, No. 1, pp. 14	Not relevant for the assessment of ED effects: This publication is considered not relevant for risk assessment of glyphosate a a glyphosate formulation was used instead of glyphosate.	The RMS agrees with the applicant's justification.
Toxicology	Shaw W.	2017	Elevated Urinary Glyphosate and Clostridia Metabolites With Altered Dopamine Metabolism in Triplets With Autistic Spectrum Disorder or Suspected Seizure Disorder: A Case Study.	Integrative medicine (Encinitas, Calif.), (2017 Feb Vol. 16, No. 1, pp. 50-57	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This is a limited case study of 3 individuals, with minimal data on glyphosate exposure.	The RMS agrees with the applicant's justification.
Toxicology	Shehata A. A. et al.	2014	Neutralization of the antimicrobial effect of glyphosate by humic acid in vitro.	Chemosphere, (2014 Jun) Vol. 104, pp. 258-61	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case c) Relevance cannot be determined: The investigation of potential effects on the gut microbiome of ruminants is not a data requirement for the approval of pesticides and suitable test protocols to assess these effects are not specified in the form of official guidance documents. Therefore, the relevance of the publication is unclear. In the absence of a suitable dossier datapoint it was allocated to point KCA 6.4 since it concerns livestock. However, it is important to note that it is not a residue study and does not provide any data on the transfer of residues from feed to food of animal origin.	The RMS agrees with the applicant's justification.
Toxicology	Solomon K.	2017	WHAT IS THE PROBLEM WITH GLYPHOSATE?	Outlooks on Pest Management, Vol. 28, No. 4, pp. 173-174, 20170801	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Review of IARC deficiencies.	The RMS agrees with the applicant's justification.

Toxicology	Solomon K. R.	2016	Glyphosate in the general population and in applicators: a critical review of studies on exposures.	Critical reviews in toxicology, (2016 Sep) Vol. 46 No. sup1, pp. 21-27	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: review, secondary source	The RMS agrees with the applicant's justification.
Toxicology	Sorahan T.	2015	Multiple myeloma and glyphosate use a re-analysis of US Agricultural Health Study (AHS) data.	International journal of environmental research an public health, (2015 Jan 28) Vol. 12, No. 2, pp. 1548-59	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5	The RMS agrees with the applicant's justification.
Toxicology	Sorahan T.	2017	Visualising and thinking and interpreting. Response to the burstyn and de roos comments on sorahan, t. multiple myeloma and glyphosate use A re-analysis of us agricultural health study (AHS) data. Int. j. environ. res. public health 2015, 12, 1548-1559	International Journal of Environmental Research and Public Health (1 Jan 2017) Vol. 14, No. 1, arn 6	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Letter to Editor	The RMS agrees with the applicant's justification.
Toxicology	Stipicevic S.	2017	Some organophosphate insecticides and herbicides	Arhiv Za Higijenu Rada i Toksikologiju, Vol. 68, No. 2, pp. A10-A11, 20170401	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Commentary on IARC evaluation.	The RMS agrees with the applicant's justification.
Toxicology	Stur E. et al.	2019	Glyphosate-based herbicides at low doses affect canonical pathways in estrogen positive and negative breast cancer cell lines.	PloS one, (2019) Vol. 14, No. 7, pp.	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate with relation to ED because AMPA was tested at concentrations in vitro that are physiologically not feasible in vivo (10 mM).	**Justification not agreed by RMS. The applicant is requested to submit an assessment of relevance and reliability for ED endpoints**
Toxicology	Suarez-Larios K. et al.	2017	Screening of Pesticides with the Potential of Inducing DSB and Successive Recombinational Repair	Journal of Toxicology, 20170101	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5.	The RMS agrees with the applicant's justification.
Toxicology	Szabo R. et al.	2017	Studies on joint toxic effects of a glyphosate herbicide (Fozat 480) and heavy metal (cadmium) on chicken embryos.	AGROFOR International Journal (2017), Vol. 2, No. 3, pp. 37-43	Not relevant for the assessment of ED effects: A glyphosate based herbicide applied to fertilized chicken eggs, not relevant to human health risk assessment.	The RMS agrees with the applicant's justification.

Toxicology	Szemeredy Geza et al.	2016	TOXICITY TEST OF INDIVIDUAL AND COMBINED TOXIC EFFECT OF HERBICIDE GLIALKA STAR AND LEAD-ACETATE ON CHICKEN EMBRYOS. Original Title: GLIALKA STAR GYOMIRTO SZER ES AZ OLOM-ACETAT EGYEDI ES INTERAKCIOS TOXICITASANAK VIZSGALATA MADAREMBRIOKBAN.	Novenyvdelem, (OCT 2016) Vol. 52, No. 10, pp. 483-487	Not relevant for the assessment of ED effects: Formulation tested via injection to chicken embryos.	The RMS agrees with the applicant's justification.
Toxicology	Tang J. et al.	2017	Ion Imbalance Is Involved in the Mechanisms of Liver Oxidative Damage in Rats Exposed to Glyphosate.	Frontiers in physiology, (2017) Vol. 8, pp. 1083	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5.	The RMS agrees with the applicant's justification.
Toxicology	Tarazona J. V. et al.	2017	Glyphosate toxicity and carcinogenicity: a review of the scientific basis of the European Union assessment and its differences with IARC.	Archives of toxicology, (2017 Aug) Vol. 91, No. 8 pp. 2723-2743	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Comparison of EU regulatory review with IARC evaluation.	The RMS agrees with the applicant's justification.
Toxicology	Tarazona J. V. et al.	2017	Response to the reply by C. J. Portier and P. Clausing, concerning our review "Glyphosate toxicity and carcinogenicity: a review of the scientific basis of the European Union assessment and its differences with IARC".	Archives of toxicology, (20170900) Vol. 91, No. 9, pp. 3199-3203	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: letter to editor, ref to Tarazona_2017.	The RMS agrees with the applicant's justification.
Toxicology	Tincher C. et al.	2017	The Glyphosate-Based Herbicide Roundup Does not Elevate Genome-Wide Mutagenesis of Escherichia coli	G3 (Bethesda, Md.), (2017 Oct 05) Vol. 7, No. 10, pp. 3331-3335	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate because a glyphosate formulation was used instead of glyphosate for in vitro testing.	The RMS agrees with the applicant's justification.
Toxicology	Townsend M. et al.	2017	Evaluation of various glyphosate concentrations on DNA damage in human Raji cells and its impact on cytotoxicity.	Regulatory toxicology and pharmacology : RTP, (2017 Apr) Vol. 85, pp. 79-85	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 5.	The RMS agrees with the applicant's justification.

Toxicology	Tribe D.	2013	Serious inadequacies regarding the pathology data presented in the paper by Seralini et al. (2012).	Food and Chemical Toxicology, (MAR 2013) Vol 53, pp. 452-457	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Letter to Editor	The RMS agrees with the applicant's justification.
Toxicology	Upadhyay J. et al.	2019	Biomarker responses (serum biochemistry) in pregnant female wistar rats and histopathology of their neonates exposed prenatally to pesticides.	Brazilian Journal of Pharmaceutical Sciences, (2019) Vol. 55. http://www.bcq.usp.br/revista_brasileira_de_cienc as.htm. ISSN: 1984-8250. E-ISSN: 2175-9790.	Not relevant for the assessment of ED effects: Formulation is not relevant for the ED risk assessment	The RMS agrees with the applicant's justification.
Toxicology	Vandenberg L. N. et al.	2017	Is it time to reassess current safety standards for glyphosate-based herbicides?.	Journal of epidemiology and community health, (2017 Jun) Vol. 71, No. 6, pp. 613-618	Not relevant for the assessment of ED effects: This publication is considered not relevant because it is not based on experimental work.	The RMS agrees with the applicant's justification.
Toxicology	Vinceti M. et al.	2017	Pesticide exposure assessed through agricultural crop proximity and risk o amyotrophic lateral sclerosis.	Environmental Health: A Global Access Science Source, (29 Aug 2017) Vol. 16, No. 1. arn. 91	Not relevant for the assessment of ED effects: No correlations wih glyphosate use and effect.	The RMS agrees with the applicant's justification.
Toxicology	Von Ehrenstei O. et al.	2019	Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: Population based case-control study	BMJ (Online) (2019) Volume 364, DOI: 10.1136/bmj.l962	Not relevant for the assessment of ED effects: This publication is not relevant for the risk assessment of glyphosate in relation to ED as the pathology investigated is not ED related (autism spectrum disorder in children).	The RMS agrees with the applicant's justification.
Toxicology	Williams G. M et al.	2016	A review of the carcinogenic potentia of glyphosate by four independent expert panels and comparison to the IARC assessment.	Critical reviews in toxicology, (2016 Sep) Vol. 46 No. sup1, pp. 3-20	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: review, secondary source	The RMS agrees with the applicant's justification.
Toxicology	Williams G. M et al.	2016	Glyphosate rodent carcinogenicity bioassay expert panel review.	Critical reviews in toxicology, (2016 Sep) Vol. 46 No. sup1, pp. 44-55	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: review, secondary source	The RMS agrees with the applicant's justification.

Toxicology	Youness E. R. et al.	2016	The protective effect of orange juice on glyphosate toxicity in adult male mice.	Journal of Chemical and Pharmaceutical Research (2016), Vol. 8, No. 3, pp. 13-28	Not relevant for the assessment of ED effects: Excessively high gavage doses to rats	The RMS agrees with the applicant's justification.
Toxicology	Yu G. C. et al.	2017	The clinical analytics of 10 patients with acute glyphosate poisoning	Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chinese journal of industrial hygiene and occupational diseases, (2017 May 20) Vol. 35, No 5, pp. 382-383	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This is a case series describing the clinical course of 10 patients who drank formulated glyphosate. All 10 patients survived the ingestion with no long-lasting sequelae. These were suicdal ingestions and should not impact re-registration.	The RMS agrees with the applicant's justification.
Toxicology	Yu Ning T. et al.	2018	Circular RNA expression profiles in hippocampus from mice with perinata glyphosate exposure.	Biochemical and biophysical research communications, (2018) Vol. 501, No. 4, pp. 838-845	Not relevant for the assessment of ED effects: This publication is considered not relevant for risk assessment of glyphosate in relation to ED as a non-ED related endpoint was investigated (circular RNA expression profiles in the hippocampus).	The RMS agrees with the applicant's justification.
Toxicology	Zhang C. et al.	2016	Health effect of agricultural pesticide use in China: implications for the development of GM crops	Scientific reports, (20161010) Vol. 6, pp. 34918	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Results likely valid for glyphosate under the exposure circumstances of the study, but not an appropriate design for chronic health effects. Short follow-up and limited exposure histories.	The RMS agrees with the applicant's justification.
Toxicology	Zhang F. et al.	2017	Study of the effect of occupational exposure to glyphosate on hepatorena function.	Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine], (2017 Jul 06) Vol. 51, No. 7, pp. 615-620	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: Very poorly described study design, methods, and analysis. Seemingly cross-sectional in time orientation precluding causal evaluation.	The RMS agrees with the applicant's justification.
Toxicology	Zhang L. et al.	2019	Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence.	Mutation research, (2019) Vol. 781, pp. 186-206	Not relevant for the assessment of ED effects: This publication is considered not relevant for the risk assessment of glyphosate in relation to ED as a non-ED related pathology was investigated in this study (non-Hodgkin lymphoma).	The RMS agrees with the applicant's justification. Considering non-ED endpoints, RMS requested a study summary in order to further justify the categorization.

						Refer to B.6.9 for an assessment of the study.
Toxicology	Zoccali C.	2017	Causal mechanism and component causes in Mesoamerican-Sri Lankan nephropathy: the moderator's view	NEPHROLOGY DIALYSIS TRANSPLANTATION, (APR 2017) Vol. 32, No. 4, pp. 607-610	Not relevant for the assessment of ED effects: no glyphosate specific information	The RMS agrees with the applicant's justification.
Toxicology	Zouaoui K. et al.	2013	Determination of glyphosate and AMPA in blood and urine from humans: about 13 cases of acute intoxication.	Forensic science international, (2013 Mar 10) Vol. 226, No. 1-3, pp. e20-5	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This is a report on a series of formulated glyphosate overdoses that found that higher blood and urine concentrations of glyphosate were associated with a poorer outcome. This is not surprising, as it reflects that patients drank a larger volume. Larger volumes of formulated product are associated with more toxicity due to the caustic nature of the surfactant, not the amount of active ingredient. All of the laboratory parameters are expected in critically ill patients. As these were suicidal ingestions, this paper should not impact re-registration.	The RMS agrees with the applicant's justification.
Toxicology	Zyoud S. H. et al.	2017	Global research production in glyphosate intoxication from 1978 to 2015: A bibliometric analysis.	Human & experimental toxicology, (2017 Oct) Vol. 36, No. 10, pp. 997-1006	Not relevant for the assessment of ED effects, but relevant for the general literature review. Assessment for the general LRR: 5.4.1 case b) Relevant but supplementary information: This article analyzes the reports of increase in glyphosate intoxications from the early 1970s-2016. Given the increase in use over the same time period it is not surprising that there has been an increase in reporting. This should not impact re-registration.	The RMS agrees with the applicant's justification.

B.6.10.2. Reference list

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
KCA 5.1.1-001	[redacted]	2020	A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats Report No.: 00050502 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N
KCA 5.1.1-002	[redacted]	1996	(^{14}C)-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat Report No.: 1413/2-1011 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.1.1-003	[redacted]	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat Report No.: [redacted]/P/4940 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.1.1-004	[redacted]	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat Report No.: [redacted]/P/4942 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
KCA 5.1.1-005	[redacted]	1996	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.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.1.1; KIIA 5.1.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.1.1-007	[redacted]	1996	Glyphosate acid: Biotransformation in the rat Report No.: [redacted]/P/5058 Document No.: [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.1.1; KIIA 5.1.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.1.1-008	[redacted] (part 1) [redacted] (part 2)	1995	Part 1: Metabolism study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley rats Part 2: Glyphosate - ADME-Study in Rats Report No.: 9202/95 (part 1), [redacted] 038/74 (part 2) Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.1.1 (OECD) Monograph 1998: EG:AIIA-5.1 Monograph Trimesium: -
KCA 5.1.1-010	[redacted]	1995	HR-001: Metabolism in the rat Report No.: [redacted]332/951256 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
KCA 5.1.1-011	[redacted]	1992	[14C]-Glyphosate: Absorption and distribution in the rat - preliminary study Report No.: 6365-676/1 Document No.: 96-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.1.1 (OECD) Monograph 1998: EG:AIIA-5.1 Monograph Trimesium: -
KCA 5.1.1-012	[redacted]	1992	[14C]-glyphosate: Absorption, distribution, metabolism and excretion in the rat, Volume I and II of II Report No.: 7006-676/2 Document No.: 100-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.1.1 (OECD) Monograph 1998: EG:AIIA-5.1 Monograph Trimesium: -
KCA 5.1.1-014	[redacted]	1988	The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration Report No.: [redacted]-7215 Document No.: M-646124-01-1 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.1.1 (OECD) Monograph 1998: EG:AIIA-5.1 Monograph Trimesium: -
KCA 5.1.1-015	[redacted] *et al.*	1988	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.: M-645024-01-1 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.1.1 (OECD) Monograph 1998: EG:AIIA-5.1 Monograph Trimesium: -

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
KCA 5.1.2-001	[redacted]	2020	Metabolic stability and profiling of [14C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison Report No.: S19-04081 Document No.: M-682096-01-1 Eurofins Agroscience Services EcoChem GmbH GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N
KCA 5.2.1-002	[redacted]	2011	Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure) Report No.: 10/218-001P Document No.: [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-006	[redacted]	2009	Glyphosate Technical: Acute Oral Toxicity Study in Rats Report No.: C22864 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	EXC	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-007	[redacted]	2009	Glyphosate: Acute Oral Toxicity Study (UDP) In Rats Report No.: 12170-08 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-008	[redacted]	2008	Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical Report No.: [redacted]-3996.305.475.07 Document No.: -	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998:

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			[redacted] GLP/GEP: Y Published: N					- Monograph Trimesium: -
KCA 5.2.1-009	[redacted]	2007	Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure) Report No.: B02755 Document No.: [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-010	[redacted]	2007	Glyphosate Technical (NUP05068): Acute Oral Toxicity Study in Rats Report No.: B02272 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-011	[redacted]	2005	Glyphosate Acid Technical - Acute Oral Toxicity Up and Down Procedure in Rats Report No.: 15274 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-012	[redacted]	1999	NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats - Limit test Report No.: 7907 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
KCA 5.2.1-013	[redacted]	1996	Glyphosate Acid: Acute Oral Toxicity Study in Rats Report No.: [redacted]/P/4660 Document No.: [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-014	[redacted]	1995	An Acute Toxicity Study of MON 0139 By Oral Administration in Mice Report No.: [redacted]-3101/XX-95-205 Document No.: M-651169-01-1 [redacted]. GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-015	[redacted]	1995	HR-001: Acute Oral Toxicity Study In Rats Report No.: [redacted]-94-0134 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-016	[redacted]	1995	HR-001: Acute Oral Toxicity Study In Mice Report No.: [redacted]-94-0133 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-017	[redacted]	1995	Final report for oral and dermal LD50 tests with [redacted] glyphosate acid technical in rats, limit test Report No.: 00917 Document No.: -	Y	N	-	COR	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998:

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			[redacted] GLP/GEP: Y Published: N					EG:AIIA-5.2.1; EG:AIIA-5.2.2 Monograph Trimesium: -
KCA 5.2.1-018	[redacted]	1995	Final report for oral and dermal LD50 tests with [redacted] glyphosate 62 % IPA in rats, limit test Report No.: 00926 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	COR	Y RAR 2017: KIIA 5.2.1; KIIA 5.2.2 (OECD) Monograph 1998: EG:AIIA-5.2.1; EG:AIIA-5.2.2 Monograph Trimesium: -
KCA 5.2.1-020	[redacted]	1994	Glyphosate Premix: Acute Oral Toxicity (Limit Test) in the Rat Report No.: 545/37 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1 Monograph Trimesium: -
KCA 5.2.1-022	[redacted]	1994	Acute oral toxicity in rats Report No.: [redacted]-94-401/R Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1 Monograph Trimesium: -
KCA 5.2.1-024	[redacted]	1992	Glyphosate technical: Acute oral toxicity (limit test) in the rat Report No.: 134/37 Document No.: - [redacted] GLP/GEP: Y	Y	N	-	BCL	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1

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			Published: N					Monograph Trimesium: -
KCA 5.2.1-025	[redacted]	1991	Assessment of acute oral toxicity of "Glyphosate technical" to mice Report No.: 12321 Document No.: 45-Gly [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1 Monograph Trimesium: -
KCA 5.2.1-026	[redacted]	1991	Acute oral toxicity study with glyphosate technical (FSG 03090 H/05 March 90) in Wistar rats Report No.: [redacted].874.AOR Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1 Monograph Trimesium: -
KCA 5.2.1-027	[redacted]	1991	Acute oral toxicity study with glyphosate technical in swiss albino mice Report No.: [redacted].875.AOM Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1 Monograph Trimesium: -
KCA 5.2.1-028	[redacted]	1990	Acute oral toxicity in the rat: Glyphosate technical Report No.: [redacted]900823B Document No.: R231 [redacted]. GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1 Monograph Trimesium: -

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KCA 5.2.1-029	[redacted]	1989	Glyphosate technical: Acute oral toxicity (limit) test in rats Report No.: 5883 Document No.: 13-Gly [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1 Monograph Trimesium: -
KCA 5.2.1-031	[redacted]	1988	Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats Report No.: 88.2053.007 Document No.: M-645904-01-1 [redacted] GLP/GEP: Y Published: Y	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.1-032	[redacted]	1987	Acute oral LD50 study of MON-8750 in Sprague-Dawley rats Report No.: 9308A Document No.: [redacted]-86-431 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1 Monograph Trimesium: -
KCA 5.2.2-001	[redacted]	2011	Glyphosate Technical - Acute Dermal Toxicity Study in Rats - Final Report Amendment 1 Report No.: 10/218-002P Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.2.2-004	[redacted]	2009	Glyphosate: Acute Dermal Toxicity Study in Rats Report No.: 12171-08 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.2-005	[redacted]	2009	Glyphosate Technical: Acute dermal toxicity study in rats Report No.: C22875 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	EXC	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.2-007	[redacted]	2008	Acute Dermal Toxicity in Wistar Hannover Rats for Glyphosate Technical Report No.: [redacted]-3996.310.456.07 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.2-008	[redacted]	2007	Glyphosate Technical (NUP 05068): Acute dermal toxicity study in rats Report No.: B02283 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.2-009	[redacted]	2007	Glyphosate technical material: Acute dermal toxicity study in rats Report No.: B02766 Document No.: [redacted]	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998:

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			GLP/GEP: Y Published: N					- Monograph Trimesium: -
KCA 5.2.2-010	[redacted]	2005	Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats - Limit Test Report No.: 15275 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.2-011	[redacted]	1996	Glyphosate Acid: Acute Dermal Toxicity in the Rat Report No.: [redacted]/P/4664 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.2-012	[redacted]	1995	HR-001: Acute dermal toxicity study in rats Report No.: 94-0154 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.2-013	[redacted]	1995	Final report for "Oral and dermal LD50 tests with [redacted] Glyphosate acid technical in rats, limit test" Report No.: 00917 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	COR	Y RAR 2017: KIIA 5.2.1 (OECD) Monograph 1998: EG:AIIA-5.2.1; EG:AIIA-5.2.2 Monograph

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								Trimesium: -
KCA 5.2.2-014	[redacted]	1995	Final report for oral and dermal LD50 tests with [redacted] glyphosate 62 % IPA in rats, limit test Report No.: 00926 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	COR	Y RAR 2017: KIIA 5.2.1; KIIA 5.2.2 (OECD) Monograph 1998: EG:AIIA-5.2.1; EG:AIIA-5.2.2 Monograph Trimesium: -
KCA 5.2.2-018	[redacted]	1992	Glyphosate technical: Acute dermal toxicity (limit test) in the rat Report No.: 134/38 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCL	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: EG:AIIA-5.2.2 Monograph Trimesium: -
KCA 5.2.2-019	[redacted]	1991	Acute dermal toxicity study with glyphosate technical (FSG 03090 H/05 March 90) in Wistar rats Report No.: [redacted].876.ADR Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: EG:AIIA-5.2.2 Monograph Trimesium: -

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KCA 5.2.2-020	[redacted]	1990	Acute dermal toxicity study in the rat: Glyphosate technical Report No.: [redacted]-900823A Document No.: R232 [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: EG:AIIA-5.2.2 Monograph Trimesium: -
KCA 5.2.2-021	[redacted]	1989	Glyphosate Technical Acute Dermal Toxicity (Limit) Test in Rats Report No.: 5884 Document No.: 14-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.2-023	[redacted]	1988	Acute dermal toxicity study of glyphosate batch/lot/NBR No. XLI-55 in New Zealand White rabbits Report No.: 88.2053.008 Document No.: M-644065-01-1 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: EG:AIIA-5.2.2 Monograph Trimesium: -
KCA 5.2.2-024	[redacted]	1987	Acute dermal toxicity study of Mon 8722 in New Zealand white rabbits Report No.: 9307A Document No.: [redacted]-86-430 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: EG:AIIA-5. 2. 2 Monograph Trimesium: -

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KCA 5.2.2-025	[redacted]	1987	Acute dermal toxicity study of Mon 8750 in New Zealand White rabbits Report No.: 9308A Document No.: R.D. No. 883 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.2 (OECD) Monograph 1998: EG:AIIA-5. 2. 2 Monograph Trimesium: -
KCA 5.2.3-001	[redacted]	2011	Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat Report No.: 11/054-004P Document No.: - [redacted] GLP/GEP: Y Published: N	Y	-	-	SYN	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.3-005	[redacted]	2009	Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat Report No.: 2743/0001 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	EXC	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.3-006	[redacted]	2009	Glyphosate - Acute Inhalation Toxicity Study in Rats Report No.: 12107-08 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.3-008	[redacted]	2007	Glyphosate technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats Report No.: B02327 Document No.: -	Y	N	-	NUF	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998:

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			[redacted] GLP/GEP: Y Published: N					- Monograph Trimesium: -
KCA 5.2.3-009	[redacted]	2005	Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats - Limit Test Report No.: 15276 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.3-010	[redacted]	2004	An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 Report No.: 3044.969 Document No.: [redacted]-2003-116 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.3-011	[redacted]	1999	NUP5a99 62 % glyphosate MUP: Acute inhalation toxicity study in rats - Limit test Report No.: 7909 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.3-012	[redacted]	1996	Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study in the Rat Report No.: [redacted]/P/4882 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.2.3-013	[redacted]	1995	HR-001: Acute inhalation toxicity study in rats Report No.: [redacted] 94-0155 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.3-016	[redacted]	1994	Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat Report No.: 545-39 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: EG:AIIA-5. 2. 3 Monograph Trimesium: -
KCA 5.2.3-020	[redacted]	1988	Acute inhalation study of MON 8750 technical Report No.: [redacted]-87-228 Document No.: [redacted]- 87147 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: EG :AIIA-5. 2. 3 Monograph Trimesium: -
KCA 5.2.3-021	[redacted]	1987	Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats Report No.: [redacted]-6582 Document No.: [redacted]-86-281 / [redacted] 86105 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.3 (OECD) Monograph 1998: EG:AIIA-5.2.3 Monograph Trimesium: -
KCA 5.2.4-001	[redacted]	2011	Glyphosate technical - Primary skin irritation study in rabbits Report No.: 10/218-006N Document No.: - [redacted]	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998:

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			GLP/GEP: Y Published: N					- Monograph Trimesium: -
KCA 5.2.4-005	[redacted]	2009	Glyphosate - Acute Dermal Irritation Study in Rabbits Report No.: 12173-08 Document No.: - [redacted]. GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.4-006	[redacted]	2008	Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical Report No.: [redacted]-3996.311.476.07 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.4-007	[redacted]	2007	Glyphosate Technical Material: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) Report No.: B02777 Document No.: [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.4-008	[redacted]	2007	Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) Report No.: B02294 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.2.4-009	[redacted]	2005	Glyphosate Acid Technical - Primary Skin Irritation Study in Rabbits Report No.: 15278 Document No.: P326 [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.4-010	[redacted]	1996	Glyphosate Acid: Skin Irritation to the Rabbit Report No.: [redacted]/P/4695 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.4-011	[redacted]	1995	HR-001: Primary Dermal irritation study in rabbits Report No.: [redacted] 95-0035 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.4-012	[redacted]	1994	Glyphosate Premix: Acute Dermal Irritation Test in the Rabbit Report No.: 545/40 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: EG:AIIA-5.2.4 Monograph Trimesium: -
KCA 5.2.4-013	[redacted]	1994	Glyphosate 360g/L: Acute dermal irritation test in the rabbit Report No.: 710/29 Document No.: - -	Y	N	-	HBX/ BPQ	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998:

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			GLP/GEP: - Published: N					EG:AIIA-5.2.6 Monograph Trimesium: -
KCA 5.2.4-015	[redacted]	1991	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 / [redacted]-GPT-SKIN [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: EG:AIIA-5.2.4 Monograph Trimesium: -
KCA 5.2.4-017	[redacted]	1990	Acute Dermal Irritation/Corrosion of Glyphosate Technical in the Rabbit (Intact and Abraded Skin) Report No.: [redacted]-900822A Document No.: [redacted]-001 / R233 [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: EG:AIIA-5.2.4 Monograph Trimesium: -
KCA 5.2.4-018	[redacted]	1989	Glyphosate Technical: Primary Skin Irritation in Rabbits Report No.: 5885 Document No.: 243268 [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: EG:AIIA-5.2.4 Monograph Trimesium: -
KCA 5.2.4-020	[redacted]	1988	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] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.2.4-021	[redacted]	1987	Primary Dermal Irritation Study of MON 8750 in New Zealand White Rabbits Report No.: [redacted]-86-431/9308A Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.4 (OECD) Monograph 1998: EG:AIIA-5.2.4 Monograph Trimesium: -
KCA 5.2.5-001	[redacted]	2011	Glyphosate technical: Acute eye irritation study in rabbits Report No.: 10/218-005N Document No.: [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.5-005	[redacted]	2009	Expert Statement Glyphosate technical: C22897: Primary eye irritation study in rabbits Report No.: C22897 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	EXC	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.5-006	[redacted]	2009	Glyphosate - Acute Eye Irritation Study in Rabbits Report No.: 12172-08 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.5-007	[redacted]	2008	Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical Report No.: [redacted]-3996.312.599.07 Document No.: -	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998:

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			[redacted] GLP/GEP: Y Published: N					- Monograph Trimesium: -
KCA 5.2.5-008	[redacted]	2007	Glyphosate Technical Material: Primary Eye Irritation Study in Rabbits Report No.: B02788 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.5-009	[redacted]	2007	Glyphosate Technical (NUP 05068): Primary Eye Irritation Study In Rabbits Report No.: B02305 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.5-010	[redacted]	2005	Glyphosate Acid Technical - Primary Eye Irritation Study in Rabbits Report No.: 15277 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.5-011	[redacted]	1997	Glyphosate Acid: Eye Irritation to the Rabbit Report No.: [redacted]/P/5138 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.2.5-012	[redacted]	1996	CHA 440: Primary eye irritation study in rabbits Report No.: 2981-96 Document No.: S9-FF81-4.C41 [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: In Addendum 1 (2000) Monograph Trimesium: -
KCA 5.2.5-013	[redacted]	1995	HR-001: Primary Eye Irritation study in rabbits Report No.: [redacted] 95-0034 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.5-019	[redacted]	1990	Acute eye irritation/corrosion of glyphosate technical in the rabbit Report No.: [redacted]-900822 Document No.: [redacted]002 / R234 [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: EG:AIIA-5.2.5 Monograph Trimesium: -
KCA 5.2.5-020	[redacted]	1989	Glyphosate technical: Primary eye irritation test in rabbits Report No.: 5886 Document No.: 243268 [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: EG:AIIA-5.2.5 Monograph Trimesium: -

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KCA 5.2.5-022	[redacted]	1988	Primary Eye Irritation Study of Glyphosate Report No.: 88. 2053. 009 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.5-024	[redacted]	1987	Primary eye irritation study of MON-8750 in New Zealand White rabbits Report No.: [redacted]-86-431/9308A Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.2.5 (OECD) Monograph 1998: EG:AIIA-5.2.5 Monograph Trimesium: -
KCA 5.2.6-001	[redacted]	2011	Glyphosate technical: Local lymph node assay in the mouse Report No.: 10/218-037E Document No.: [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.6-004	[redacted]	2009	Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs - Maximisation-Test Report No.: C22908 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	EXC	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.6-008	[redacted]	2007	Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test Report No.: B02316 Document No.: -	Y	N	-	NUF	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998:

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			[redacted] GLP/GEP: Y Published: N					- Monograph Trimesium: -
KCA 5.2.6-009	[redacted]	2007	Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay in the Mouse) Report No.: GM8048-REG Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.6-010	[redacted]	2006	Glyphosate Technical: Skin Sensitisation in the Guinea Pig - Magnusson and Kligman Maximisation method Report No.: [redacted]-05/0218 Document No.: 2060/009 [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.6-011	[redacted]	2005	Glyphosate acid technical - Dermal Sensitisation in Guinea Pigs (Buehler Method) Report No.: 15279 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.6-012	[redacted]	1996	Glyphosate Acid: Skin Sensitisation to the Guinea Pig Report No.: [redacted]/P/4699 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.2.6-013	[redacted]	1995	HR-001: Dermal sensitisation study in guinea pigs Report No.: [redacted] 95-0036 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.2.6-015	[redacted]	1994	Glyphosate Premix: Magnusson & Kligman Maximisation Study in the Guinea Pig Report No.: 545/42 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: EG:AIIA-5.2.6 Monograph Trimesium: -
KCA 5.2.6-019	[redacted] *et al.*	1991	Luxan Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig Report No.: 349/11 Document No.: R 199 [redacted] GLP/GEP: Y Published: N	Y	N	-	LUX	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: EG:AIIA-5.2.6 Monograph Trimesium: -
KCA 5.2.6-020	[redacted]	1989	Glyphosate technical: Magnusson-Kligman maximisation test in guinea pigs Report No.: 5887 Document No.: 243268 [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.2.6 (OECD) Monograph 1998: EG :AIIA-5.2.6 Monograph Trimesium: -
KCA 5.2.6-023	Lindberg, T. et al.	2020	An integrated transcriptomic- and proteomic-based approach to evaluate the human skin sensitization potential of glyphosate and its commercial agrochemical formulations Report No.: doi.org/10.1016/j.jprot.2020.103647	N	N	--	LIT	N

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			Document No.: - Journal of Proteomics GLP/GEP: N Published: Y					
KCA 5.3.1-001	[redacted]	1991	28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) Report No.: [redacted].881.28 DDR Document No.: TOXI-881/1991 [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.3.1 (OECD) Monograph 1998: EG:AIIA-5.3.1 Monograph Trimesium: -
KCA 5.3.1-004	[redacted] *et al.*	1989	Glyphosate: 4 Week Dietary Toxicity Study in Rats Report No.: 5626 Document No.: 437462 [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.3.1 (OECD) Monograph 1998: EG:AIIA-5.3.1 Monograph Trimesium: -
KCA 5.3.1-005	[redacted]	1989	Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats Report No.: [redacted]-8921 Document No.: [redacted]-88-272/[redacted] 88181 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-5.3.1 Monograph Trimesium: -
KCA 5.3.1-006	[redacted]	1978	A Four Week Pilot Study with Glyphosate in Mice Report No.: 77-2110 Document No.: [redacted]-77-418 [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-5.3.1 Monograph Trimesium: -

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KCA 5.3.1-008	[redacted]	1982	Range finding study of MON 0139 and isopropylamine administered orally to dogs Report No.: [redacted]-2155 Document No.: [redacted]-81-032 / 810036 [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.3.1 (OECD) Monograph 1998: EG:AIIA-5.3.1 Monograph Trimesium: -
KCA 5.3.1-009	Gao, H. *et al.*	2019	Activation of the N-methyl-d-aspartate receptor is involved in glyphosate-induced renal proximal tubule cell apoptosis. Report No.: doi.org/10.1002/jat.3795; E-ISSN: 1099-1263; L-ISSN: 0260-437X. Document No.: - Journal of applied toxicology : JAT, (2019) .Volume: 39; Issue: 8, pp. 1096-1107 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.3.2-001	[redacted]	1996	First Revision to Glyphosate Acid: 90 Day Oral Feeding Study in Rats Report No.: [redacted]/P/1599 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.2-003	[redacted] *et al.*	1996	Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study In The Rat Report No.: 434/016 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.3.2-004	[redacted]	1995	HR-001: 13-week Subchronic Oral Toxicity Study in Rats Report No.: [redacted] 94-0138 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.2-005	[redacted]	1993	90 Day Range Feeding Study of Glyphosate in Rats (Vol. 1) Report No.: 011-0001 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: E:G:AIIA-5.3.2 Monograph Trimesium: -
KCA 5.3.2-008	[redacted]	1992	90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) Report No.: [redacted]-882 90 OR Document No.: TOXI-882/1991 [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: EG:AIIA-5.3.2 Monograph Trimesium: -
KCA 5.3.2-011	[redacted] *et al.*	1991	Glyphosate - 13-week dietary toxicity study in rats Report No.: 7136 Document No.: 437876 [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: EG:AIIA-5.3.2 Monograph Trimesium: -
KCA 5.3.2-013	[redacted]	1989	Glyphosate Technical: 90 Day Oral Toxicity study in the Rat Report No.: [redacted]-891002 Document No.: [redacted]-401 [redacted].	Y	N	-	BCL	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998:

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			GLP/GEP: Y Published: N					EG:AIIA-5.3.2 Monograph Trimesium: -
KCA 5.3.2-014	[redacted]	1987	90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats Report No.: [redacted]-7375 Document No.: [redacted]-86-351/[redacted] 86128 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: EG:AIIA-5.3.2 Monograph Trimesium: -
KCA 5.3.2-017	[redacted]	1995	HR-001: 13-week Oral Subchronic Toxicity Study in Mice Report No.: [redacted] 94-0136 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.2-018	[redacted] *et al.*	1991	Glyphosate: 13-week dietary toxicity study in rats Report No.: 7024 Document No.: 437918 [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: EG:AIIA-5.3.2 Monograph Trimesium: -
KCA 5.3.2-019	[redacted]	1979	A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice Report No.: 77-2111 Document No.: - [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-5.3.2 Monograph Trimesium: -

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KCA 5.3.2-020	[redacted]	2007	Glyphosate Technical: 13-Week Toxicity Study By Oral Route (Capsule) In Beagle Dogs Report No.: 29646[redacted] Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.3.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.2-021	[redacted]	1999	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) Report No.: 1816 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.2-025	[redacted]	1996	First Revision to Glyphosate Acid: 90-Day Oral Toxicity Study in Dogs Report No.: [redacted]/P/1802 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.3.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.2-027	[redacted]	1996	HR-001: 13-week Oral Subchronic Toxicity Study in Dogs Report No.: [redacted] 94-0158 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.3.2 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.3.2-029	[redacted]	1983	Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs Report No.: 810166 Document No.: [redacted]-81-368 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.3.3 (OECD) Monograph 1998: EG:AIIA-5.3.2 Monograph Trimesium: -
KCA 5.3.2-031	[redacted]	2007	Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs Report No.: 29647 [redacted] Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.3.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.2-032	[redacted]	1997	HR-001: 12-Month Oral Chronic Toxicity Study in Dogs Report No.: [redacted] 94-0157 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.3.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.2-033	[redacted]	1996	Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs Report No.: [redacted]/P/5079 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.3.4 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.3.2-035	[redacted]	1990	Glyphosate: 52 Week Oral Toxicity Study in Dogs Report No.: 7502 Document No.: 642675 [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.3.4 (OECD) Monograph 1998: EG:AIIA-5.5 Monograph Trimesium: -
KCA 5.3.2-036	[redacted]	1985	Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs Report No.: [redacted]-4965 Document No.: [redacted]-83-137 / 830116 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.3.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.3-001	[redacted]	1996	Glyphosate Acid: 21-Day Dermal Toxicity Study in Rats Report No.: [redacted]/P/4985 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 5.3.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.3.3-003	[redacted] *et al.*	1993	Glyphosate: 3 Week Toxicity Study in Rats with Dermal Administration Report No.: 7839 Document No.: 450881 [redacted] GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 5.3.1 (OECD) Monograph 1998: EG :AIIA-5.3.3 Monograph Trimesium: -
KCA 5.3.3-004	[redacted]	1994	Glyphosate technical ([redacted]): Repeated dose twenty-eight- Day dermal toxicity study in rabbits (Part 1, Study Report) Report No.: [redacted] 214/94 Document No.: -	Y	N	-	GTF	Y RAR 2017: KIIA 5.3.1; KIIA 5.3.7 (OECD)

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			[redacted] GLP/GEP: Y Published: N					Monograph 1998: EG:AIIA-5.3.3 Monograph Trimesium: -
KCA 5.3.3-010	Mesnage, R. *et al.*	2018	Comparison of transcriptome responses to glyphosate, isoxaflutole, quizalofop-p-ethyl and mesotrione in the HepaRG cell line. Report No.: doi.org/10.1016/j.toxrep.2018.08.005; E-ISSN: 2214-7500; L-ISSN: 2214-7500 Document No.: PMC-PMC6098220 Toxicology reports, (2018) Vol. 5, pp. 819-826. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.3.3-011	Kumar, S. *et al.*	2014	Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation Report No.: doi.org/10.1016/j.tox.2014.08.008; E-ISSN: 1879-3185.; L-ISSN: 0300-483X. Document No.: NLM-NIHMS629285; NLM-PMC4195794; PMC-PMC4195794; MID-NIHMS629285 Toxicology, (2014) Vol. 325, pp. 42-51. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.4.1-001	[redacted]	2014	Glyphosate: Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli* Report No.: 41401854 Document No.: - Harlan Laboratories Ltd. GLP/GEP: Y Published: N	N	N	-	ALB	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.4.1-002	[redacted]	2012	Reverse mutation assay using Bacteria (*Salmonella typhimurium*) with Glyphosate tech. Report No.: 126159 Document No.: - BSL Bioservice Scientific Laboratories GmbH GLP/GEP: Y Published: N	N	N	-	INA	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-004	[redacted]	2010	*Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine Report No.: 1332300 Document No.: C88226 Harlan Cytotest Cell Research GmbH (Harlan CCR) GLP/GEP: Y Published: N	N	N	-	HAG	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-005	[redacted]	2010	Reverse Mutation Assay using Bacteria (*Salmonella typhimurium*) with Glyphosate TC Report No.: 101268 Document No.: - BSL Bioservice Scientific Laboratories GmbH GLP/GEP: Y Published: N	N	N	-	HAG	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-007	[redacted]	2009	Glyphosate technical - *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay Report No.: 1264500 Document No.: - Harlan Cytotest Cell Research GmbH (Harlan CCR) GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-009	[redacted]	2007	*Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with glyphosate technical (NUP-05068) Report No.: 1061401 Document No.: -	N	N	-	NUF	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998:

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			RCC Cytotest Cell Research GmbH (RCC-CCR) GLP/GEP: Y Published: N					- Monograph Trimesium: -
KCA 5.4.1-010	[redacted]	2007	*Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with glyphosate technical (NUP-05070) Report No.: 1061402 Document No.: - RCC Cytotest Cell Research GmbH (RCC-CCR) GLP/GEP: Y Published: N	N	N	-	NUF	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-011	[redacted]	2007	*Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with glyphosate technical (NUP-05067) Report No.: 1061403 Document No.: - RCC Cytotest Cell Research GmbH (RCC-CCR) GLP/GEP: Y Published: N	N	N	-	NUF	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-013	[redacted]	1996	Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli* Report No.: CTL/P/4874 Document No.: Central Toxicology Laboratory GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-014	[redacted]	1996	Technical glyphosate: Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli* Report No.: 434/014 Document No.: - SafePharm Laboratories Limited GLP/GEP: Y Published: N	N	N	-	NUF	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.4.1-015	[redacted]	1995	HR-001: Reverse Mutation Test Report No.: IET 94-0142 Document No.: - The Institute of Environmental Toxicology GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-018	[redacted]	1993	Mutagenicity - *Salmonella typhimurium* reverse mutation assay (Ames Test) Report No.: 887-MUT.AMES Document No.: TOXI-887/1993 ES-GPT-MUT.AMES Rallis India Limited, Toxicology Department GLP/GEP: Y Published: N	N	N	-	ADM	Y RAR 2017: - Monograph 1998: EG:AIIA-5.4.1 Monograph Trimesium: -
KCA 5.4.1-020	[redacted]	1991	Mutagenicity test: Ames *Salmonella* assay with Glyphosate, batch 206-JaK-25-1 Report No.: 12323 Document No.: - Scantox A/S GLP/GEP: Y Published: N	N	N	-	FMC	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: EG:AIIA-5.4.1 Monograph Trimesium: -
KCA 5.4.1-024	[redacted] *et al.*	1978	The report of mutagenic study with bacteria for CP 67573 Report No.: ET-78-241 Document No.: - The Institute of Environmental Toxicology, Kodaira, Tokyo 187, Japan GLP/GEP: N Published: N	N	N	-	BCS	Y RAR 2017: KIIA 5.4.1 (OECD) Monograph 1998: EG:AIIA-5.4.1 Monograph Trimesium: -
KCA 5.4.1-025	[redacted]	1998	Glyphosate Acid: *In Vitro* Cytogenetic Assay In Human Lymphocytes Report No.: CTL/P/6050 Document No.: - Central Toxicology Laboratory	N	N	-	SYN	Y RAR 2017: KIIA 5.4.2 (OECD) Monograph 1998:

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			GLP/GEP: Y Published: N					- Monograph Trimesium: -
KCA 5.4.1-026	[redacted]	1996	Technical glyphosate: Chromosome aberration test in CHL cells *in vitro* Report No.: 434/015 Document No.: - Safepharm Laboratories Limited GLP/GEP: Y Published: N	N	N	-	NUF	Y RAR 2017: KIIA 5.4.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-027	[redacted]	1995	HR-001: *In vitro* cytogenicity test Report No.: IET 94-0143 Document No.: - The Institute of Environmental Toxicology, 2-772 Suzuki-cho, Kodaira, Tokyo 1 87, J apan GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 5.4.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-028	[redacted]	1995	Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) Report No.: 141918 Document No.: - NOTOX B.V. GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 5.4.2 (OECD) Monograph 1998: EG:AIIA-5.4.1 Monograph Trimesium: -
KCA 5.4.1-030	[redacted]	1998	Glyphosate Acid: L5178Y TK+/- Mouse Lymphoma Gene Mutation Assay Report No.: CTL/P/4991 Document No.: - Central Toxicology Laboratory GLP/GEP: Y	N	N	-	SYN	Y RAR 2017: KIIA 5.4.3 (OECD) Monograph 1998: - Monograph

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			Published: N					Trimesium: -
KCA 5.4.1-031	[redacted]	1991	Mutagenicity test: *In vitro* Mammalian Cell Gene Mutation Test with Glyphosate, batch 206-JaK-25-1 Report No.: 12325 Document No.: - Scantox A/S GLP/GEP: Y Published: N	N	N	-	FMC	Y RAR 2017: KIIA 5.4.2 (OECD) Monograph 1998: EG:AIIA-5.4.1 Monograph Trimesium: -
KCA 5.4.1-032	[redacted]	1983	CHO/HGPRT Gene Mutation Assay with Glyphosate Report No.: ML-83-155 Document No.: - Monsanto Agricultural Company Environmental Health Laboratory GLP/GEP: N Published: N	N	N	-	BCS	Y RAR 2017: KIIA 5.4.2; KIIA 5.4.3; KIIA 4.4.4 (OECD) Monograph 1998: EG:AIIA-5.4.1 Monograph Trimesium: -
KCA 5.4.1-035	[redacted]	1995	HR-001: DNA Repair Test (Rec-Assay) Report No.: IET 94-0141 Document No.: - The Institute of Environmental Toxicology GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 5.4.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.1-040	[redacted]	2021	Glyphosate: V79 HPRT Gene Mutation Assay Report No.: 8841968 Document No.: - Covance Laboratories Limited GLP/GEP: Y	N	Y	First submission in EU	GRG	N

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			Published: N					
KCA 5.4.1-041	[redacted]	2021	Micronucleus Test in Human Lymphocytes in vitro Report No.: 8841969 Document No.: - Covance Laboratories Limited GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 5.4.2-001	[redacted]	2012	Micronucleus Test of Glyphosate TGAI in Mice Report No.: 485-1-06-4696 Document No.: [redacted]-0112-6927-003 [redacted] GLP/GEP: Y Published: N	Y	N	--	COR	Y RAR 2017: -- Monograph 1998: -- AIR2 Doc J: J-II/11 / 05 Monograph Trimesium: --
KCA 5.4.2-002	[redacted]	2012	Glyphosate technical - Micronucleus assay in bone marrow cells of the mouse Report No.: 1479200 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	--	SYN	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: -- Monograph Trimesium: --
KCA 5.4.2-004	[redacted]	2010	First amendment to the report: Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice Report No.: [redacted]-3996.402.395.07 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	HAG	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.4.2-005	[redacted]	2008	Glyphosate Technical - Micronucleus Assay in Bone Marrow Cells of the Mouse Report No.: 1158500 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.2-007	[redacted]	2006	Glyphosate Technical: Micronucleus Test In The Mouse Report No.: 2060/014 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.2-008	[redacted]	1999	A micronucleus study in mice for glifosate técnico [redacted] Report No.: [redacted]-G12.79/99 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4.2-009	[redacted]	1996	Glyphosate Acid: Mouse Bone Marrow Micronucleus Test Report No.: [redacted]/P/4954 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.4.2-010	[redacted]	1993	Glyphosate technical: Mutagenicity - Micronucleus Test in Swiss Albino Mice Report No.: 889-MUT.MN Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: EG:AIIA-5.4.2 Monograph Trimesium: -
KCA 5.4.2-012	[redacted]	1991	Mutagenicity test: Micronucleus test with Glyphosate, batch 206-JaK-25-1 Report No.: 12324 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: EG:AIIA-5.4.2 Monograph Trimesium: -
KCA 5.4.2-015	[redacted]	1994	Genetic toxicology: In vivo mammalian bone marrow cytogenetic test – Chromosomal analysis Report No.: 890-MUT-CH.AB Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: EG:AIIA-5.4.2 Monograph Trimesium: -
KCA 5.4.2-016	[redacted]	1983	*In Vivo* Bone Marrow Cytogenetics Study of Glyphosate in Sprague-Dawley Rats Report No.: 830083 Document No.: [redacted]-645019-01-1 [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.4.4 (OECD) Monograph 1998: EG:AIIA-5.4.2 Monograph Trimesium: -
KCA 5.4.3-001	[redacted]	1992	Dominant lethal test in Wistar rats Report No.: TOXI: 888-DLT Document No.: - [redacted]	Y	N	-	ADM	Y RAR 2017: KIIA 5.4.6 (OECD) Monograph 1998:

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			GLP/GEP: Y Published: N					EG:AIIA-5.4.3 Monograph Trimesium: -
KCA 5.4.3-002	[redacted]	1992	Dominant lethal test in Wistar rats, Appendix 1-45 Report No.: TOXI: 888-DLT Document No.: TOXI-888/1992 [redacted]GPT-DLT [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.4.6 (OECD) Monograph 1998: EG:AIIA-5.4.3 Monograph Trimesium: -
KCA 5.4.3-003	[redacted]	1992	Dominant lethal test in Wistar rats, Appendix 46-53 Report No.: TOXI: 888-DLT Document No.: TOXI-888/1992 [redacted]GPT-DLT [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.4.6 (OECD) Monograph 1998: EG:AIIA-5.4.3 Monograph Trimesium: -
KCA 5.4.3-004	[redacted]	1982	Mutagenic testing of glyphosate in rat by dominant lethal test Report No.: - Document No.: - [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-5.4.2 Monograph Trimesium: -
KCA 5.4.3-005	[redacted] *et al.*	1980	Dominant lethal mutagenicity assay with technical Glyphosate in mice Report No.: 401-064 Document No.: M-643921-01-1 [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.4.6 (OECD) Monograph 1998: EG:AIIA-5.4.3 Monograph Trimesium: -

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KCA 5.4-001	Adler-Flindt, S. *et al.*	2019	Comparative cytotoxicity of plant protection products and their active ingredients Report No.: doi.org/10.1016/j.tiv.2018.10.020 ISSN: 0887-2333 Document No.: - Toxicology *In Vitro*, (2019) Vol. 54, pp. 354-366. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.4-002	Ilyushina, N. *et al.*	2018	Maximum tolerated doses and erythropoiesis effects in the mouse bone marrow by 79 pesticides' technical materials assessed with the micronucleus assay. Report No.: doi.org/10.1016/j.toxrep.2018.12.006 ISSN: 2214-7500 Document No.: - Toxicology Reports (2019) Vol. 6, pp. 105-110 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.4-003	Nagy, K. *et al.*	2019	Comparative cyto- and genotoxicity assessment of glyphosate and glyphosate-based herbicides in human peripheral white blood cells. Report No.: doi.org/10.1016/j.envres.2019.108851; E-ISSN: 1096-0953; L-ISSN: 0013-9351 Document No.: - Environmental research, (2019) Vol. 179, No. 108851. pp. 1-7 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.4-004	de Almeida, L. K. S. *et al.*	2018	Moderate levels of glyphosate and its formulations vary in their cytotoxicity and genotoxicity in a whole blood model and in human cell lines with different estrogen receptor status. Report No.: DOI: 10.1007/s13205-018-1464-z; ISSN: 2190-572X Document No.: - 3 Biotech (2018), Volume 8:438, Number 10, pp. 1-15. GLP/GEP: N Published: Y	N	N	-	LIT	N

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KCA 5.4-006	Santovito, A. *et al.*	2018	*In vitro* evaluation of genomic damage induced by glyphosate on human lymphocytes. Report No.: doi.org/10.1007/s11356-018-3417-9; E-ISSN: 1614-7499; L-ISSN: 0944-1344 Document No.: - Environmental science and pollution research international, (2018) Vol. 25, No. 34, pp. 34693-34700. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.4-007	Kasuba, V. *et al.*	2017	Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line. Report No.: DOI 10.1007/s11356-017-9438-y; E-ISSN: 1614-7499; L-ISSN: 0944-1344 Document No.: - Environmental science and pollution research international, (2017) Vol. 24, No. 23, pp. 19267-19281. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.4-008	Kwiatkowska, M. *et al.*	2017	DNA damage and methylation induced by glyphosate in peripheral blood mononuclear cells (*in vitro* study) Report No.: doi.org/10.1016/j fct.2017.03.051 ISSN: 0278-6915 Document No.: - Food and chemical toxicology (2017) GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.4-009	Suárez-Larios, K. *et al.*	2017	Screening of Pesticides with the Potential of Inducing DSB and Successive Recombinational Repair Report No.: DOI: 10.1155/2017/3574840 Document No.: - Journal of Toxicology, Vol. 2017, pp. 1-9. GLP/GEP: N Published: Y	N	N	-	LIT	N

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KCA 5.4-011	Roustan, A. *et al.*	2014	Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation Report No.: E-ISSN: 1879-1298 L-ISSN: 0045-6535 Document No.: - Chemosphere, (2014) Vol. 108, pp. 93-100. GLP/GEP: N Published: Y	N	n	-	LIT	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4-012	Mañas, F. *et al.*	2013	Oxidative stress and comet assay in tissues of mice administered glyphosate and AMPA in drinking water for 14 days. Report No.: ISSN: 1666-0390 Document No.: - BAG - Journal of Basic and Applied Genetics, (2013) Vol. 24, No. 2, pp. 67-75. GLP/GEP: N Published: Y	N	n	-	LIT	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.4-013	Koller, V.J. *et al.*	2012	Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. Report No.: DOI 10.1007/s00204-012-0804-8; E-ISSN: 1432-0738; L-ISSN: 0340-5761. Document No.: - Archives of toxicology, (2012) Vol. 86, No. 5, pp. 805-13. GLP/GEP: N Published: Y	N	n	-	LIT	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.5-001	[redacted] *et al.*	2009	Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat Report No.: 2060-0012 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.5.2 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.5-002	[redacted]	2001	Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats Report No.: [redacted]/PR1111 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.5.2; KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.5-004	[redacted]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats Report No.: [redacted] 94-0150 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.5.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.5-005	[redacted]	1996	Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats Report No.: 886.C.C-R Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.5.2 (OECD) Monograph 1998: EG:AIIA-5.5 Monograph Trimesium: -
KCA 5.5-006	[redacted]	1996	Glyphosate Acid: One Year Dietary Toxicity Study in Rats Report No.: [redacted]/P/5143 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.5.1; KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.5-007	[redacted] *et al.*	1993	Glyphosate - 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) - Vol I Report No.: 7867 Document No.: 153-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.5.2 (OECD) Monograph 1998: EG:AIIA-5.5 Monograph Trimesium: -
KCA 5.5-008	[redacted] *et al.*	1993	Glyphosate - 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) - Vol II Report No.: 7867 Document No.: 153- GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.5.2 (OECD) Monograph 1998: EG:AIIA-5.5 Monograph Trimesium: -
KCA 5.5-009	[redacted] *et al.*	1993	Glyphosate - 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) - Vol III Report No.: 7867 Document No.: 153- GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.5.2 (OECD) Monograph 1998: EG:AIIA-5.5 Monograph Trimesium: -
KCA 5.5-010	[redacted]	1990	Chronic study of glyphosate administered in feed to Albino rats Report No.: [redacted]-10495 Document No.: M-651388-02-1 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.5.2 (OECD) Monograph 1998: EG:AIIA-5.5 Monograph Trimesium: -
KCA 5.5-012	[redacted] *et al.*	2009	Glyphosate Technical: Dietary Carcinogenicity Study in the Mouse Report No.: 2060-0011 Document No.: - [redacted]	Y	N	-	NUF	Y RAR 2017: KIIA 5.5.3 (OECD) Monograph 1998:

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			GLP/GEP: Y Published: N					- Monograph Trimesium: -
KCA 5.5-013	[redacted]	2010	Historical Incidence of Malignant lymphoma in CD-1 Mouse Report No.: ASB2015-2531 Document No.: - [redacted] GLP/GEP: N Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 5.5 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.5-014	[redacted]	2011	Glyphosate Technical: Dietary carcinogenicity study in the mouse – Amendment Report No.: 2060-0011 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.6.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.5-015	[redacted]	2011	Assessment and further discussion on relevance of perceived elevation in testicular atrophy for SafePharm project number 2060/0011 (Glyphosate technical: mouse oncogenicity study) Report No.: 2060-0011 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.6.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.5-016	[redacted]	2001	Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice Report No.: TOXI: 1559.CARCI-M Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: KIIA 5.5.3 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.5-018	[redacted]	1997	HR-001: 18-Month Oral Oncogenicity Study in Mice Report No.: [redacted] 94-0151 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.5.3 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.5-020	[redacted]	1993	Glyphosate: 104-week dietary carcinogenicity study in mice Report No.: 7793 Document No.: 154-Gly [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.5.3 (OECD) Monograph 1998: EG:AIIA-5.5 Monograph Trimesium: -
KCA 5.5-023	[redacted]	1983	A chronic feeding study of glyphosate (Roundup® technical) in mice Report No.: 77-2061 Document No.: M-646425-01-1 [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.5.3 (OECD) Monograph 1998: EG:AIIA-5. 5 Monograph Trimesium: -
KCA 5.5-026	Crump, K. *et al.*	2020	Accounting for Multiple Comparisons in Statistical Analysis of the Extensive Bioassay Data on Glyphosate Report No.: https://doi.org/10.1093/toxsci/kfaa039 Document No.: - Toxicological Sciences, 2020 Mar 19 [Epub ahead of print] GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.5-027	Portier, C.J.	2020	A comprehensive analysis of the animal carcinogenicity data for glyphosate from chronic exposure rodent carcinogenicity studies Report No.: doi.org/10.1186/s12940-020-00574-1 Document No.: -	N	N	-	LIT	N

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			Portier Environmental Health (2020) 19:18 GLP/GEP: N Published: Y					
KCA 5.5-028	Wozniak, E. *et al.*	2019	Glyphosate affects methylation in the promoter regions of selected tumor suppressors as well as expression of major cell cycle and apoptosis drivers in PBMCs (*in vitro* study). Report No.: doi.org/10.1016/j.tiv.2019.104736; E-ISSN: 1879-3177; L-ISSN: 0887-2333 Document No.: - Toxicology *in vitro* (2019) Vol. 63, Issue: 104736. pp. 1-7. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.5-029	Biserni, M. *et al.*	2019	Quizalofop-p-Ethyl Induces Adipogenesis in 3T3-L1 Adipocytes. Report No.: doi: 10.1093/toxsci/kfz097; ISSN: 1096-0929; L-ISSN: 1096-0929 Document No.: - Toxicological sciences (2019) Volume: 170. No. 2, pp. 452–461 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.5-030	Crump, K.	2019	The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Report No.: DOI: 10.1111/risa.13440; E-ISSN: 1539-6924; L-ISSN: 0272-4332. Document No.: - Risk analysis : an official LITation of the Society for Risk Analysis (2019) GLP/GEP: N Published: Y	N	N	-	LIT	N

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KCA 5.5-031	Duforestel, M. *et al.*	2019	Glyphosate Primes Mammary Cells for Tumorigenesis by Reprogramming the Epigenome in a TET3-Dependent Manner. Report No.: doi: 10.3389/fgene.2019.00885; ISSN: 1664-8021; L-ISSN: 1664-8021 Document No.: PMC-PMC6777643 Frontiers in genetics, (2019) Vol. 10, pp. 885. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.5-032	Hao, Y. *et al.*	2019	Roundup-Induced AMPK/mTOR-Mediated Autophagy in Human A549 Cells. Report No.: doi.org/10.1021/acs.jafc.9b04679; E-ISSN: 1520-5118; L-ISSN: 0021-8561 Document No.: - Journal of agriculture and food chemistry (2019) Vol. 67, No. 41, pp 11364-11372 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.5-033	Pahwa, M. *et al.*	2019	Glyphosate use and associations with non-Hodgkin lymphoma major histological sub-types: findings from the North American Pooled Project. Report No.: doi:10.5271/sjweh.3830; E-ISSN: 1795-990X; L-ISSN: 0355-3140 Document No.: - Scandinavian journal of work, environment & health, (2019). Vol. 45, Issue. 6, pp. 600-609 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.5-034	Wang, L. *et al.*	2019	Glyphosate induces benign monoclonal gammopathy and promotes multiple myeloma progression in mice. Report No.: doi.org/10.1186/s13045-019-0767-9; E-ISSN: 1756-8722; L-ISSN: 1756-8722 Document No.: - Journal of hematology & oncology, (2019) Vol. 12, No. 1, pp. 70.	N	N	-	LIT	N

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			GLP/GEP: N Published: Y					
KCA 5.5-035	Andreotti, G. *et al.*	2018	Glyphosate Use and Cancer Incidence in the Agricultural Health Study Report No.: doi: 10.1093/jnci/djx233; ISSN: 0027-8874 Document No.: - JNCI-Journal of the National Cancer Institute, (2018) Vol. 110, No. 5, pp. 509-516. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.5-036	Presutti, R. *et al.*	2016	Pesticide exposures and the risk of multiple myeloma in men: An analysis of the North American Pooled Project. Report No.: DOI: 10.1002/ijc.30218; ISSN: 0020-7136; E-ISSN: 1097-0215 Document No.: - International Journal of Cancer, (2016) Vol. 139, No. 8, pp. 1703-1714. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.5-037	Sorahan, T.	2015	Multiple myeloma and glyphosate use: a re-analysis of US Agricultural Health Study (AHS) data. Report No.: doi:10.3390/ijerph120201548; E-ISSN: 1660-4601; L-ISSN: 1660-4601 Document No.: NLM-PMC4344679; PMC-PMC4344679 International journal of environmental research and LIT health, (2015) Vol. 12, No. 2, pp. 1548-59. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.5-038	Alavanja, M.C.R. *et al.*	2013	Increased Cancer Burden Among Pesticide Applicators and Others Due to Pesticide Exposure Report No.: doi:10.3322/caac.21170 Document No.: - A Cancer Journal for Clinicians, (2013) Vol. 63, No. 2, pp. 120–142	N	N	--	LIT	N

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			GLP/GEP: N Published: Y					
KCA 5.5-040	De Roos, A.J. *et al.*	2003	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men Report No.: doi: 10.1136/oem.60.9.e11 Document No.: - Occupational & Environmental Medicine, (2003) Vol. 60, No. 9, e11 GLP/GEP: N Published: Y	N	N	--	LIT	N
KCA 5.5-041	De Roos, A.J. *et al.*	2005	Cancer Incidence among Glyphosate-Exposed Pesticide Applicators in the Agricultural Health Study Report No.: doi: 10.1289/ehp.7340 Document No.: - Environmental Health Perspective (2005) Vol. 113, No. 1, pp. 49–54 GLP/GEP: N Published: Y	N	N	--	LIT	N
KCA 5.5-047	Lee, W.J. *et al.*	2005	Agricultural pesticide use and risk of glioma in Nebraska, United States Report No.: DOI: 10.1136/oem.2005.020230 Document No.: - Occupational & Environtal Medicine, (2005) Vol. 62, No. 11, pp. 786-792 GLP/GEP: N Published: Y	N	N	--	LIT	N
KCA 5.5-048	McDuffie, H.H. *et al.*	2001	Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health Report No.: - Document No.: - Cancer Epidemiology, Biomarkers & Prevention, (2001) Vol. 10, No. 11, pp. 1155-1163 GLP/GEP: N	N	N	--	LIT	N

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			Published: Y					
KCA 5.5	Schinasi, L. & Leon, M.E.	2014	Non-Hodgkin Lymphoma and Occupational Exposure to Agricultural Pesticide Chemical Groups and Active Ingredients: A Systematic Review and Meta-Analysis Report No.: - - Document No.: Int. J. Environ. Res. Public Health 2014; 11:4449-4527 - Int. J. Environ. Res. Public Health 2014; 11:4449-4527 GLP/GEP: N Published: Y	-	N	-	LIT	-
KCA 5.6.1-001	[redacted] et al.	2007	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat Report No.: 2060/0013 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.6.1 Monograph 1998: - Monograph Trimesium: -
KCA 5.6.1-002	[redacted] et al.	2008	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat (First amendment to report) Report No.: 2060/0013 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.6.1 Monograph 1998: - Monograph Trimesium: -

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KCA 5.6.1-003	[redacted] et al.	2008	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat (Second amendment to report) Report No.: 2060/0013 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.6.1 Monograph 1998: - Monograph Trimesium: -
KCA 5.6.1-004	[redacted]	2000	Glyphosate acid: Multigeneration reproduction toxicity study in rats Report No.: [redacted]/P/6332 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.6.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.6.1-005	[redacted]	1997	HR-001: A two-generation reproduction study in rats Report No.: [redacted] 96-0031 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.6.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.6.1-007	[redacted] et al.	1992	The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat (Vol. 1) Report No.: [redacted] 47/911129 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.6.1 (OECD) Monograph 1998: EG:AIIA-5.6.1 Monograph Trimesium: -

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KCA 5.6.1-008	[redacted] et al.	1992	The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat (Vol. 2) Report No.: [redacted] 47/911129 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.6.1 (OECD) Monograph 1998: EG:AIIA-5.6.1 Monograph Trimesium: -
KCA 5.6.1-010	[redacted]	1990	Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats Report No.: [redacted]-10387 Document No.: M-643937-01-1 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.6.1 (OECD) Monograph 1998: EG:AIIA-5.6.1 Monograph Trimesium: -
KCA 5.6.1-015	Gorga, A. et al.	2020	In vitro effects of glyphosate and Roundup on Sertoli cell physiology Report No.: doi.org/10.1016/j.tiv.2019.104682 E-ISSN: 1879-3177; L-ISSN: 0887-2333 Document No.: - Toxicological sciences: (2012) Vol. 127, No. 2, pp. 391-402. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.6.1-016	Manservisi, F. et al.	2019	The Ramazzini Institute 13-week pilot study glyphosate-based herbicides administered at human-equivalent dose to Sprague Dawley rats: effects on development and endocrine system Report No.: PMC-PMC6413565 doi.org/10.1186/s12940-019-0453-y E-ISSN: 1476-069X; L-ISSN: 1476-069X Document No.: - Environmental health: a global access science source, (2019) Vol. 18, No. 1, pp. 15. GLP/GEP: N Published: Y	Y	N	-	LIT	N

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KCA 5.6.1-017	Pham, T.H. et al.	2019	Perinatal Exposure to Glyphosate and a Glyphosate-Based Herbicide Affect Spermatogenesis in Mice Report No.: doi: 10.1093/toxsci/kfz039 E-ISSN: 1096-0929; L-ISSN: 1096-0929 Document No.: - Toxicological sciences (2019) Vol. 169, No. 1, pp. 260-271. GLP/GEP: N Published: Y	Y	N	-	LIT	N
KCA 5.6.1-018	Ren, X. et al.	2019	Effects of chronic glyphosate exposure to pregnant mice on hepatic lipid metabolism in offspring Report No.: doi.org/10.1016/j.envpol.2019.07.074 E-ISSN: 1873-6424; L-ISSN: 0269-7491 Document No.: - Environmental pollution (2019) Vol. 254, No. 112906. pp. 1-8. GLP/GEP: N Published: Y	Y	N	-	LIT	N
KCA 5.6.1-019	Zhang, J.W. et al.	2019	The toxic effects and possible mechanisms of glyphosate on mouse oocytes Report No.: doi.org/10.1016/j.chemosphere.2019.124435 E-ISSN: 1879-1298; L-ISSN: 0045-6535 Document No.: - Chemosphere, (2019) Vol. 237, Issue: 124435, pp. 1-10. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.6.1-020	Johansson, H.K. et al.	2018	Exposure to a glyphosate-based herbicide formulation, but not glyphosate alone, has only minor effects on adult rat testis Report No.: doi.org/10.1016/j reprotox.2018.09.008 E-ISSN: 1873-1708; L-ISSN: 0890-6238 Document No.: - Reproductive toxicology (Elmsford, N.Y.), (2018) Vol. 82, pp. 25-31. GLP/GEP: N Published: Y	N	N	-	LIT	N

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KCA 5.6.1-021	Panzacchi, S. et al.	2018	The Ramazzini Institute 13-week study on glyphosate-based herbicides at humanequivalent dose in Sprague Dawley rats: study design and first in-life endpoints evaluation Report No.: doi.org/10.1186/s12940-018-0393-y ISSN: 1476-069X Document No.: - Environmental Health, (2018) Vol. 17, Issue:1, pp. 52/1-52/13. GLP/GEP: N Published: Y	Y	N	-	LIT	N
KCA 5.6.1-022	Perego, M.C.	2017	Evidence for direct effects of glyphosate on ovarian function: glyphosate influences steroidogenesis and proliferation of bovine granulosa but not theca cells in vitro Report No.: DOI 10.1002/jat.3417 E-ISSN: 1099-1263; L-ISSN: 0260-437X Document No.: - Journal of applied toxicology (2017) Vol. 37, No. 6, pp. 692-698. GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: KCA 5.8.2 Monograph 1998: - Monograph Trimesium: -
KCA 5.6.1-023	Dai, P. et al.	2016	Effect of glyphosate on reproductive organs in male rat Report No.: doi.org/10.1016/j.acthis.2016.05.009 E-ISSN: 1618-0372; L-ISSN: 0065-1281 Document No.: - Acta histochemica, (2016) Vol. 118, No. 5, pp. 519-26. GLP/GEP: N Published: Y	Y	N	-	LIT	Y RAR 2017: KIIA 5.10 Monograph 1998: - Monograph Trimesium: -
KCA 5.6.1-024	Forgacs, A.L. et al.	2012	BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants Report No.: doi:10.1093/toxsci/kfs121 E-ISSN: 1096-0929; L-ISSN: 1096-0929 Document No.: - Toxicological sciences: (2012) Vol. 127, No. 2, pp. 391-402. GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.6.2-001	[redacted]	2002	Glyphosate Acid: Developmental Toxicity Study in the Rat (Including Amendment 001 to Glyphosate Acid: Developmental Toxicity Study in the Rat) Report No.: [redacted]/P/4819 Document No.: M-301383-01-1 [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.6.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.6.2-002	[redacted]	1995	HR-001: Teratogenicity Study in Rats Report No.: [redacted] 94-0152 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.6.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.6.2-003	[redacted]	1991	The effect of glyphosate on pregnancy of the rat (incorporates preliminary investigation) Report No.: [redacted] 43 & 41/90716 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.6.10 (OECD) Monograph 1998: EG:AIIA-5.6.2 Monograph Trimesium: -
KCA 5.6.2-008	[redacted]	1980	Teratology study in rats Report No.: 401-054 Document No.: M-644179-02-1 [redacted] GLP/GEP: N (pre GLP) Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.6.10 (OECD) Monograph 1998: EG:AIIA-5.6.2 Monograph Trimesium: -

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KCA 5.6.2-009	[redacted]	1996	Glyphosate acid: Developmental toxicity study in the rabbit Report No.: [redacted]/P/5009 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.6.11 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.6.2-010	[redacted]	1996	Glyphosate technical: Oral gavage teratology study in the rabbit Report No.: 434/020 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.6.11 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.6.2-011	[redacted]	1995	HR-001: A Teratogenicity Study in Rabbits Report No.: [redacted] 94-0153 Document No.: M-301383-01-1 [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.6.11 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.6.2-014	[redacted] et al.	1991	The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations) Report No.: [redacted] 45 & 39 & 40/901303 Document No.: 74-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.6.11 (OECD) Monograph 1998: EG:AIIA-5.6.2 Monograph Trimesium: -
KCA 5.6.2-015	[redacted] et al.	1991	The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations) Report No.: [redacted] 45 & 39 & 40/901303 Document No.: 74-GLY	Y	N	-	FMC	Y RAR 2017: KIIA 5.6.11 (OECD) Monograph 1998:

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			[redacted] GLP/GEP: Y Published: N					EG:AIIA-5.6.2 Monograph Trimesium: -
KCA 5.6.2-018	[redacted] et al.	1980	Technical Glyphosate: Pilot Teratology Study in Rabbits Report No.: 401-055 Document No.: - [redacted] GLP/GEP: N (pre GLP) Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.6.11 (OECD) Monograph 1998: EG:AIIA-5.6.2 Monograph Trimesium: -
KCA 5.7.1-001	[redacted]	1996	Glyphosate acid: Acute neurotoxicity study in rats Report No.: [redacted]/P/4866 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.7.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.7.1 -002	[redacted] *et al.*	2006	Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat Report No.: 2060-0010 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N
KCA 5.7.1-003	[redacted]	1996	Glyphosate Acid: Subchronic Neurotoxicity Study In Rats Report No.: [redacted]/P/4867 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.7.4 (OECD) Monograph 1998: - Monograph Trimesium: -

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
KCA 5.7.2-001	[redacted]	1996	Glyphosate Acid: Acute delayed neurotoxicity study in the domestic hen Report No.: [redacted]/C/3122 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.7 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.7-001	Martinez, A. *et al.*	2019	Effects of glyphosate and aminomethylphosphonic acid on an isogeneic model of the human blood-brain barrier. Report No.: doi.org/10.1016/j.toxlet.2018.12.013; E-ISSN: 1879-3169; L-ISSN: 0378-4274 Document No.: - Toxicology letters, (2019) Vol. 304, pp. 39-49. GLP/GEP: N Published: Y	N	n	-	LIT	N
KCA 5.7-002	Martinez, M.A. *et al.*	2018	Neurotransmitter changes in rat brain regions following glyphosate exposure Report No.: doi.org/10.1016/j.envres.2017.10.051; E-ISSN: 1096-0953; L-ISSN: 0013-9351 Document No.: - Environmental research, (2018) Vol. 161, pp. 212-219. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.7-003	Chorfa, A. *et al.*	2013	Specific pesticide-dependent increases in α-synuclein levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines Report No.: doi:10.1093/toxsci/kft076; E-ISSN: 1096-0929; L-ISSN: 1096-0929 Document No.: - Toxicological sciences (2013) Vol. 133, No. 2, pp. 289-97. GLP/GEP: N Published: Y	N	N	-	LIT	RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -

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CA 5.6	Ait-Bali, Y. et al.	2020	Pre- and postnatal exposure to glyphosate-based herbicide causes behavioral and cognitive impairments in adult mice: evidence of cortical ad hippocampal dysfunction. Report No.: - - Document No.: Archives of toxicology, (2020) Vol. 94, No. 5, pp. 1703-1723 - Archives of toxicology, (2020) Vol. 94, No. 5, pp. 1703-1723 GLP/GEP: N Published: Y	-	N	-	LIT	-
KCA 5.8.1-002	[redacted]	1996	AMPA: Acute Oral Toxicity Study In Mice Report No.: [redacted] 96-0075 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.1-003	[redacted]	1993	AMPA: Acute oral toxicity (limit) test in rats Report No.: 8763 Document No.: 117-Gly [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG :AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-004	[redacted]	1991	Assessment of acute oral toxicity of (N-methyl-N-phosphonomethyl)glycine to rats Report No.: 12837 Document No.: 75-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG:AIIA-5.8.1 Monograph Trimesium: -

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KCA 5.8.1-005	[redacted]	1988	Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat Report No.: [redacted]/P/2266 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.1-008	[redacted]	1993	AMPA: Acute Dermal Toxicity (Limit) Test in Rats Report No.: 8764 Document No.: 118-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG :AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-012	[redacted]	1993	AMPA: Magnusson-Kligman Maximisation Test in Guinea Pigs Report No.: 8765 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG:AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-014	[redacted] *et al.*	1993	4 Week Dose Range Finding Study in Rats with Administration by Gavage Report No.: 7803 Document No.: 148-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG:AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-016	[redacted] *et al.*	1993	13 Week Toxicity Study in Rats with Administration by Gavage Report No.: 7866 Document No.: 152-GLY [redacted]	Y	N	-	FMC	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998:

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			GLP/GEP: Y Published: N					EG:AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-017	[redacted] *et al.*	1979	90-Day Subacute Rat Toxicity Study Report No.: 401-050 Document No.: M-644184-01-1 [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG:AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-018	[redacted]	1991	90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA Report No.: [redacted]-50173 Document No.: M-645465-01-1 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG:AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-019	[redacted]	1996	AMPA: Reverse Mutation Test Report No.: [redacted] 96-0076 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.1-020	[redacted]	1993	Mutagenicity test: Ames Salmonella Test with AMPA, batch 286-JRJ-73-4 Report No.: 13269 Document No.: 145-GLY Scantox A/S GLP/GEP: Y Published: N	N	N	-	FMC	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG:AIIA-5.8.1 Monograph Trimesium: -

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KCA 5.8.1-021	[redacted]	1988	Aminomethyl Phosphonic Acid: An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli* Report No.: CTL/P/2206 Document No.: - Central Toxicology Laboratory GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.1-045	[redacted]	2021	Aminomethylphosphonic acid: Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli* Report No.: - Document No.: - - GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 5.8.1-046	[redacted]	2021	Aminomethylphosphonic acid: V79 HPRT Gene Mutation Assay Report No.: 8441963 Document No.: - Covance Laboratories Limited GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 5.8.1-047	[redacted]	2021	Aminomethylphosphonic acid: Micronucleus Test in Human Lymphocytes *in vitro* Report No.: - Document No.: - - GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 5.8.1-026	[redacted]	1993	Mutagenicity test: Micronucleus test with AMPA, batch 286-JRJ-73-4 Report No.: 13268 Document No.: 146-GLY [redacted] GLP/GEP: Y	N	N	-	FMC	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG:AIIA-5.8.1

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			Published: N					Monograph Trimesium: -
KCA 5.8.1-027	[redacted]	1993	Mouse Micronucleus Study of AMPA Report No.: [redacted]-13243 Document No.: [redacted]-90170 / [redacted]-90-404 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG :AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-028	[redacted]	1992	AMPA: Teratogenicity study in rats Report No.: 7891 Document No.: 124-Gly [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG:AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-029	[redacted]	1991	A dose range-finding developmental toxicity study of AMPA in rats Report No.: [redacted]-50146 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-5.8.1 Monograph Trimesium: -
KCA 5.8.1-030	[redacted]	1991	A developmental toxicity study of AMPA in rats Report No.: [redacted]-50159 Document No.: M-645464-01-1 [redacted]. GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.8 (OECD) Monograph 1998: EG:AIIA-5.8.1 Monograph Trimesium: -

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Submittted as KCA 6.7.1-001 Reported at KCA 5.8.1	Knoell Germany GmbH	2020	(Q)SAR and read-across genotoxicity evaluation of Glyphosate and seven metabolites, using VEGA v1.1.5b22, DEREK Nexus v6.0.1, Toxtree v3.1.0 and OECD QSAR Toolbox v4.4 Report No.: 110054/QSAR/1 Document No.: knoell Germany GmbH GLP/GEP: N Published: N	N			GRG	N
Submittted as KCA 6.7.1-001 Reported at KCA 5.8.1	Knoell Germany GmbH	2020	Supplementary information for (Q)SAR and read-across genotoxicity evaluation of Glyphosate and seven metabolites, using VEGA v1.1.5b22, DEREK Nexus v6.0.1, Toxtree v3.1.0 and OECD QSAR Toolbox v4.4 Report No.: 110054/QSAR/1 Document No.: knoell Germany GmbH GLP/GEP: N Published: N	N			GRG	N
KCA 5.8.1-032	[redacted]	2007	IN-EYE252: Acute Oral Toxicity Study in Rats - Up-and-Down Procedure Report No.: [redacted]-22229 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N
KCA 5.8.1-033	[redacted]	2008	IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats Report No.: [redacted]-23316 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N

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KCA 5.8.1-034	[redacted]	2007	IN-EY252: Bacterial Reverse Mutation Assay Report No.: AB47BT.503.BTL Document No.: DuPont-22227 BioReliance GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N:
KCA 5.8.1-035	[redacted]	2007	IN-EY252: *In Vitro* Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes Report No.: AB47BT.341.BTL Document No.: DuPont-22225 BioReliance GLP/GEP: Y Published: N	N	Y	First submission in the EU	GRG	N
KCA 5.8.1-036	[redacted]	2007	IN-EY252: *In Vitro* Mammalian Cell Gene Mutation Test (CHO/HGPRT) Report No.: DuPont-22224 Document No.: - E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences AND Critical Path Services, LLC, Analytical Laboratory GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 5.8.1-037	[redacted]	2007	IN-EY252: Mouse Bone Marrow Micronucleus Test Report No.: [redacted]-22226 Document No.: [redacted]-22226 [redacted] GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N

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KCA 5.8.1-038	[redacted]	2004	Mass Balance, Metabolism, and Pharmacokinetics of [^{14}C]N-acetylglyphosate Following Administration of a Single Oral Dose to Rats Report No.: [redacted] 7535-100 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N
KCA 5.8.1-039	[redacted]	2004	Acute Oral Toxicity Study in Rats with N-Acetyl-Glyphosate, Sodium Salt (Acute Toxic Class Method) Report No.: 7535-103 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N
KCA 5.8.1-040	[redacted]	2007	IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats Report No.: [redacted]-19008 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N
KCA 5.8.1-041	[redacted]	2004	*Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a confirmatory Assay with N-Acetyl-Glyphosate Report No.: 7535-101 Document No.: VER04-COV-03 Covance Laboratories Inc. GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N

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KCA 5.8.1-042	[redacted]	2004	Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells Report No.: 7535-102 Document No.: VER04-COV-02 Covance Laboratories Inc. GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 5.8.1-043	[redacted]	2006	IN-MCX20: *In Vitro* Mammalian Cell Gene Mutation Test (CHO/HGPRT) Report No.: DuPont-20155 Document No.: - E.I. du Pont de Nemours and Company, HaskellSM Laboratory for Health and Environmental Sciences GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 5.8.1-044	[redacted]	2006	IN-MCX20: Mouse Bone Marrow Micronucleus Test Report No.: [redacted]-20154 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	Y	First submission in EU	GRG	N
KCA 5.8.2-001	[redacted]	2012	Glyphosate - A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice Report No.: [redacted]-50393 Document No.: M-651162-01-1 [redacted] GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 5.8.2-002	[redacted]	2010	An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats Report No.: [redacted]-50361 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.2-003	[redacted]	1996	Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat Report No.: [redacted]/P/5160 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN / BCS	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.2-004	[redacted]	1996	Glyphosate Technical: Pharmacology Screening Study in the Rat Report No.: 434/021 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.2-005	[redacted]	1992	Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study Report No.: [redacted] 90-0149/ET-92-15 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: EG:AIIA-5.8.2 Monograph Trimesium: -
KCA 5.8.2-006	Anonymous	1988	Toxicodinamic study of glyphosate in rat Report No.: - Document No.: - [redacted].	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG: AIIA-5 .1

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			[redacted] GLP/GEP: N Published: N					Monograph Trimesium: -
KCA 5.8.2-007	[redacted]	1988	Synergism and potentiation in rats of Glyphosate (tech.) of [redacted] Report No.: - Document No.: - [redacted] GLP/GEP: N Published: N	Y	N	-	BCL	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: EG :AIIA-5.8.2 Monograph Trimesium: -
KCA 5.8.2-008	[redacted] *et al.*	1987	The acute toxicity of glyphosate in female goats Report No.: 80006 Document No.: [redacted]-80-450 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: EG :AIIA-5.8.2 Monograph Trimesium: -
KCA 5.8.2-009	[redacted] *et al.*	1987	The acute oral toxicity of the isopropylamine salt of glyphosate (MON 0139) in female goats Report No.: 80007 Document No.: [redacted]-80-451 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: EG :AIIA-5.8.2 Monograph Trimesium: -
KCA 5.8.2-011	[redacted]	1987	Irritating effect of Glyphosate, Surfactant and Roundup on Stomach and small Intestine in Dogs Report No.: 2309496 Document No.: - [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 5.9; KIIA 5.10; KIIIA1 7.6.3 (OECD) Monograph 1998: EG:AIIA-5. 9

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								Monograph Trimesium: -
KCA 5.8.2-014	[redacted]	2012	Glyphosate acid - *In Vitro* Absorption through Abraded Rabbit Skin using [14C]-glyphosate Report No.: JV2182-REG Document No.: - Dermal Technology Laboratory Ltd. GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 5.3.1; KIIIA1 7.6.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.3-001	[redacted]	2012	Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay Report No.: 6500V-100334ARB Document No.: CTX-11-026 CeeTox, Inc. GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.3-003	[redacted]	2012	Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay Report No.: 6500V-100334ERB Document No.: CTX-11-029 CeeTox, Inc. GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.3-004	[redacted]	2012	Glyphosate: Human Recombinant Aromatase Assay Report No.: 6500V-100334AROM Document No.: CTX-11-027 CeeTox, Inc. GLP/GEP: Y	Y	N	-	GTF	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: -

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
			Published: N					Monograph Trimesium: -
KCA 5.8.3-005	[redacted]	2012	A Uterotrophic Assay of Glyphosate Administrered Orally in Ovariectomized Rats Report No.: [redacted]-843002 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.3-006	[redacted]	2012	A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats Report No.: [redacted]-843003 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.3-007	[redacted]	2012	A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats Report No.: [redacted]-843005 Document No.: - [redacted] Biotechnics, LLC GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.3-008	[redacted]	2012	A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats - Volume 1 of 2 - Report No.: [redacted]-843007 Document No.: - [redacted]	Y	N	-	GTF	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph

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			[redacted] GLP/GEP: Y Published: N					Trimesium: -
KCA 5.8.3-009	Hecker, M. *et al.*	2011	The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter- laboratory validation study Report No.: DOI 10.1007/s11356-010-0396-x Document No.: - Environ Sci Pollut Res (2011) 18:503–515 GLP/GEP: N Published: Y	N	N	-	LIT	Y RAR 2017: KIIA 5.10 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 5.8.3-011	[redacted]	2020	(Q)SAR screening on endocrine disrupting potential of Glyphosate under the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 Report No.: 110517-1 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 5.8.3-012	Gigante, P. *et al.*	2018	Glyphosate affects swine ovarian and adipose stromal cell functions. Report No.: doi.org/10.1016/j.anireprosci.2018.05.023; E-ISSN: 1873-2232; L-ISSN: 0378-4320 Document No.: - Animal reproduction science, (2018) Vol. 195, pp. 185-196. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.8.3-013	Vanlaeys, A. *et al.*	2018	Formulants of glyphosate-based herbicides have more deleterious impact than glyphosate on TM4 Sertoli cells. Report No.: doi.org/10.1016/j.tiv.2018.01.002; E-ISSN: 1879-3177; L-ISSN: 0887-2333 Document No.: -	N	N	-	LIT	N

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			Toxicology *in vitro* (2018) Vol. 52, pp. 14-22 GLP/GEP: N Published: Y					
KCA 5.8.3-014	Mesnage, R. *et al.*	2017	Evaluation of estrogen receptor alpha activation by glyphosate-based herbicide constituents. Report No.: doi.org/10.1016/j fct.2017.07.025; E-ISSN: 1873-6351; L-ISSN: 0278-6915. Document No.: - Food and chemical toxicology (2017) Vol. 108, No. Pt A, pp. 30-42. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.8.3-015	Thongprakaisang, S. *et al.*	2013	Glyphosate induces human breast cancer cells growth via estrogen receptors. Report No.: doi.org/10.1016/j fct.2013.05.057; E-ISSN: 1873-6351; L-ISSN: 0278-6915. Document No.: - Food and chemical toxicology (2013) Vol. 59, pp. 129-36. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.8.3-016	Brennan, J.C. *et al.*	2015	Development of a Recombinant Human Ovarian (bg1) Cell Line Containing Estrogen Receptor α and β for Improved Detection of Estrogenic/Antiestrogenic Chemicals Report No.: DOI: 10.1002/etc.3146 Document No.: - Environmental Toxicology and Chemistry, Vol. 35, No. 1, pp. 91–100, 2016 GLP/GEP: N Published: Y	N	N	--	LIT	N

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KCA 5.8.3-018	Defarge, N. *et al.*	2016	Co-Formulants in Glyphosate-Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels Report No.: doi:10.3390/ijerph13030264 Document No.: - Int. J. Environ. Res. Public Health 2016, 13, 264 GLP/GEP: N Published: Y	N	N	--	LIT	N
KCA 5.6-001	Ganesan, S. *et al.*	2020	Report title Absence of glyphosate induced effects on ovarian folliculogenesis and steroidogenesis Report No.: 'DOI: 10.1016/j reprotox.2020.06.011 Document No.: - Reproductive Toxicology, (2020) 96, 156 164 GLP/GEP: N Published: Y	N	N	--	LIT	N
KCA 5.8.3-020	Gastiazoro, M.P. *et al.*	2020	Glyphosate induces epithelial mesenchymal transition-related changes in human endometrial Ishikawa cells via estrogen receptor pathway Report No.: -- Document No.: -- Molecular and cellular endocrinology, (2020), Vol. 510, Art. No. 110841 GPL/GEP: N Published: N	N	N	--	LIT	
KCA 5.8.3-021	Xia, Y. *et al.*	2020	The endoplasmic reticulum stress and related signal pathway mediated the glyphosate-induced testosterone synthesis inhibition in TM3 cells Report No.: DOI: 10.1016/j.envpol.2020.113949 Document No.: -- Environmental Pollution, (2020) 260, 113949 GLP/GEP: N Published: Y	N	N	--	LIT	N

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KCA 5.9-001	Connolly, A. *et al.*	2018	Characterising glyphosate exposures among amenity horticulturists using multiple spot urine samples. Report No.: doi.org/10.1016/j.ijheh.2018.06.007; E-ISSN: 1618-131X; L-ISSN: 1438-4639 Document No.: - International journal of hygiene and environmental health, (2018) Vol. 221, No. 7, pp. 1012-1022. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.9-002	Connolly, A. *et al.*	2019	Exploring the half-life of glyphosate in human urine samples. Report No.: doi.org/10.1016/j.ijheh.2018.09.004; E-ISSN: 1618-131X; L-ISSN: 1438-4639. Document No.: - International journal of hygiene and environmental health, (2019) Vol. 222, No. 2, pp. 205-210. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.9-003	Connolly, A. *et al.*	2017	Exposure assessment using human biomonitoring for glyphosate and fluroxypyr users in amenity horticulture. Report No.: doi.org/10.1016/j.ijheh.2017.06.008; ISSN: 1618-131X; L-ISSN: 1438-4639. Document No.: - International journal of hygiene and environmental health, (2017) Vol. 220, No. 6, pp. 1064-1073. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.9-004	Connolly, A. *et al.*	2018	Glyphosate in Irish adults - A pilot study in 2017. Report No.: doi.org/10.1016/j.envres.2018.04.025; E-ISSN: 1096-0953; L-ISSN: 0013-9351 Document No.: - Environmental research, (2018) Vol. 165, pp. 235-236. GLP/GEP: N Published: Y	N	N	-	LIT	N

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KCA 5.9-005	Connolly, A. *et al.*	2019	Evaluating Glyphosate Exposure Routes and Their Contribution to Total Body Burden: A Study Among Amenity Horticulturalists. Report No.: doi: 10.1093/annweh/wxy104; E-ISSN: 2398-7316; L-ISSN: 2398-7308. Document No.: - Annals of work exposures and health, (2019) Vol. 63, No. 2, pp. 133-147. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.9-006	Conrad, A. *et al.*	2017	Glyphosate in German adults - Time trend (2001 to 2015) of human exposure to a widely used herbicide Report No.: doi.org/10.1016/j.ijheh.2016.09.016100898843; E-ISSN: 1618-131X; L-ISSN: 1438-4639. Document No.: - International journal of hygiene and environmental health, (2017) Vol. 220, No. 1, pp. 8-16. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.9-007	Kongtip, P. *et al.*	2017	Glyphosate and Paraquat in Maternal and Fetal Serums in Thai Women. Report No.: doi.org/10.1080/1059924X.2017.1319315; E-ISSN: 1545-0813; L-ISSN: 1059-924X. Document No.: - Journal of agromedicine, (2017) Vol. 22, No. 3, pp. 282-289. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.9-008	McGuire, M. K. *et al.*	2016	Glyphosate and aminomethylphosphonic acid are not detectable in human milk. Report No.: doi: 10.3945/ajcn.115.126854; ISSN: 1938-3207; L-ISSN: 0002-9165. Document No.: - The American journal of clinical nutrition, (2016) Vol. 103, No. 5, pp. 1285-90.	N	N	-	LIT	N

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			GLP/GEP: N Published: Y					
KCA 5.9-009	Sierra-Diaz, E. *et al.*	2019	Urinary pesticide levels in children and adolescents residing in two agricultural communities in Mexico Report No.: doi:10.3390/ijerph16040562; ISSN: 1660-4601 Document No.: - International Journal of Environmental Research and LIT Health, (2019) Vol. 16, No. 4, pp. 562. GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.9-010	Steinborn, A. *et al.*	2016	Determination of Glyphosate Levels in Breast Milk Samples from Germany by LC-MS/MS and GC-MS/MS. Report No.: doi.org/10.1021/acs.jafc.5b05852; E-ISSN: 1520-5118; L-ISSN: 0021-8561 Document No.: - Journal of agriculture and food chemistry (2016) Vol. 64, No. 6, pp 1414-1421 GLP/GEP: N Published: Y	N	N	-	LIT	N
KCA 5.9-011	Trasande, L. *et al.*	2020	Glyphosate exposures and kidney injury biomarkers in infants and young children. Report No.: doi.org/10.1016/j.envpol.2019.113334; E-ISSN: 1873-6424; L-ISSN: 0269-7491 Document No.: - Environmental pollution (2020) Vol. 256, Issue: 113334, pp. 1-8. GLP/GEP: N Published: Y	N	N	-	LIT	N

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KCA 5.9.7-12	Acquavella, J. F. et al.	1999	Human ocular effects from self-reported exposures to Roundup® herbicides Report No.: - - Document No.: Human & Experimental Toxicology (1999), Vol. 18, pp. 479-486 - Human & Experimental Toxicology (1999), Vol. 18, pp. 479-486 GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-13	Acquavella, J.F. et al.	2004	Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study Report No.: - - Document No.: Environmental health perspectives (2004), Vol. 112, Issue 3, 321-326; https://doi.org/10.1289/ehp.6667 - Environmental health perspectives (2004), Vol. 112, Issue 3, 321-326; https://doi.org/10.1289/ehp.6667 GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-14	Bando, H. et al.	2010	An extreme hyperkalemia in a patient with a new glyphosate potassium herbicide poisoning: Report of a case (Original in Japanese + English translation, 6 pages) Report No.: - - Document No.: Chudoku Kenkyu 23 (3):246-249. (Japanese)	-	N	-	LIT	Y

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			- Chudoku Kenkyu 23 (3):246-249. (Japanese) GLP/GEP: N Published: Y					
KCA 5.9.7-15	Barbosa, E.R. et al.	2001	Parkinsonism After Glycine-Derivate Exposure Movement Disorders Report No.: - - Document No.: Movement Disorders, (2011), Vol. 16, No. 3, pp. 565-568 - Movement Disorders, (2011), Vol. 16, No. 3, pp. 565-568 GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-16	Bradberry, S.M. et el.	2004	Glyphosate poisoning Report No.: - - Document No.: Toxicol Rev. 2004;23(3):159-167. doi:10.2165/00139709-200423030-00003 - Toxicol Rev. 2004;23(3):159-167. doi:10.2165/00139709-200423030-00003 GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-17	Brunetti, R. M. et al.	2019	Electrocardiographic abnormalities associated with acute glyphosate toxicity Report No.: - - Document No.: HeartRhythm Case Reports, (2019), Vol. 6, Issue 2, pp. 63-66	-	N	-	LIT	N

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			https://doi.org/10.1016/j.hrcr.2019.10.014 - HeartRhythm Case Reports, (2019), Vol. 6, Issue 2, pp. 63-66 https://doi.org/10.1016/j.hrcr.2019.10.014 GLP/GEP: N Published: Y					
KCA 5.9.7-18	Chang, C.Y. et al.	1999	Clinical impact of upper gastrointestinal tract injuries in glyphosate-surfactant oral intoxication Report No.: - - Document No.: Human & Experimental Toxicology, (1999) Vol. 18, pp. 475-478 - Human & Experimental Toxicology, (1999) Vol. 18, pp. 475-478 GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-19	Goldstein, D.A. et al.	1999	Pneumonitis and Herbicide Exposure Report No.: - - Document No.: CHEST, (1999), Vol. 116, No. 4, pp. 1139 - CHEST, (1999), Vol. 116, No. 4, pp. 1139 GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-20	Goldstein, D.A. et al.	2002	An analysis of glyphosate data from the California Environmental Protection Agency Pesticide Illness Surveillance Program Report No.: - -	-	N	-	LIT	Y

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			Document No.: Journal of Toxicology. Clinical Toxicology 40(7):885-892 (2013) DOI: 10.1081/clt-120016960 - Journal of Toxicology. Clinical Toxicology 40(7):885-892 (2013) DOI: 10.1081/clt-120016960 GLP/GEP: N Published: Y					
KCA 5.9.7-21	Kamijo, Y. et al.	2012	Glyphosate surfactant herbicide products containing glyphosate potassium salt can cause fatal hyperkalemia if ingested in massive amounts Report No.: - - Document No.: Clinical Toxicology 50:159 (2012). DOI: 10.3109/15563650.2011.648747. - Clinical Toxicology 50:159 (2012). DOI: 10.3109/15563650.2011.648747. GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-22	Khot, R. et al.	2018	Glyphosate poisoning with acute fulminant hepatic failure Report No.: - - Document No.: Asia Pacific Journal of Medical Toxicology 7(3):86-88 (2018) DOI: 10.22038/apjmt.2018.11984 - Asia Pacific Journal of Medical Toxicology 7(3):86-88 (2018) DOI: 10.22038/apjmt.2018.11984	-	N	-	LIT	N

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			GLP/GEP: N Published: Y					
KCA 5.9.7-23	Lee, H. L. et al.	2000	Clinical presentations and prognostic factors of a glyphosate-surfactant herbicide intoxication: a review of 131 cases Report No.: - - Document No.: Academic Emergency Medicine 7(8):906-10 (2000) DOI: 10.1111/j.1553-2712.2000.tb02069.x - Academic Emergency Medicine 7(8):906-10 (2000) DOI: 10.1111/j.1553-2712.2000.tb02069.x GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-24	Pushnoy, L. A. et al.	1998	Herbicide (Roundup) Pneumonitis Report No.: - - Document No.: CHEST 1998; 114:1769-1771 - CHEST 1998; 114:1769-1771 GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-25	Sawada, Y. & Nagai, Y.	1987	Roundup poisoning --- its clinical observation possible involvement of surfactant (Original + English translation, 12 pages) Report No.: - - Document No.: Journal of Clinical and Experimental Medicine	-	N	-	LIT	Y

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			(IGAKU NO AYUMI) - Vol. 143 No.l p. 25-27 (1987.10.3) - Journal of Clinical and Experimental Medicine (IGAKU NO AYUMI) - Vol. 143 No.l p. 25-27 (1987.10.3) GLP/GEP: N Published: Y					
KCA 5.9.7-26	Sawada, Y. et al.	1988	Probable toxicity of surface-active agent in commercial herbicide containing glyphosate. Report No.: - - Document No.: The Lancet 1988, p.299 - The Lancet 1988, p.299 GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-27	Tominack, R. et al.	1989	Clinical Management of ROUNDUP Herbicide Exposure Report No.: - - Document No.: The Japanese Journal of Toxicology, 2, 1989, 187-192. - The Japanese Journal of Toxicology, 2, 1989, 187-192. GLP/GEP: N Published: Y	-	N	-	LIT	Y
KCA 5.9.7-28	Wang, G. et al.	2011	Letter to the Editor Parkinsonism after chronic occupational exposure to glyphosate Report No.: - - Document No.: Parkinsonism and Related Disorders 17 (2011) 486–487	-	N	-	LIT	Y

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			- Parkinsonism and Related Disorders 17 (2011) 486–487 GLP/GEP: N Published: Y					
KCA 5.9.7-29	Zheng, Q. et al.	2018	Correspondence Reversible Parkinsonism induced by acute exposure glyphosate Report No.: - - Document No.: Parkinsonism and Related Disorders 50 (2018) 121 - Parkinsonism and Related Disorders 50 (2018) 121 GLP/GEP: N Published: Y	-	N	-	LIT	Y