1. Information on the study

Data point:	CA 5.6
Report author	Perego M.C. *et al.*
Report year	2017
Report title	Evidence for direct effects of glyphosate on ovarian function: glyphosate influences steroidogenesis and proliferation of bovine granulosa but not theca cells *in vitro*
Document No	J. Appl. Toxicol. 2017; 37: 692–698
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restriction

2. Assessment and conclusion

Assessment and conclusion by applicant:

In this *in vitro* study, glyphosate had minimal effects on granulosa cells (GC). In the presence of FSH only, glyphosate had no effect on GC cell viability or on progesterone or estradiol production. In the presence of FSH and IGF1, glyphosate reduced GC proliferation without a dose-response at 0.5 and 5 μg ml^{-1} but not at lower test concentrations (0.01 and 0.3 μg ml^{-1}) and did not affect progesterone production or CYP19A1 and CYP11A1 mRNA expression; estradiol production was reduced at 5 μg ml^{-1} only (not at lower test concentrations). Without FSH or IGF1, 1.7 μg ml^{-1} of glyphosate slightly increased GC proliferation in response to serum (≤11%).

Glyphosate at 5 μg ml^{-1} had no effect on the theca cell (TC) proliferation or the production of progesterone or androstenedione.

Overall, with the exception of slight, non-dose-related alterations in GC proliferation under different test conditions, this study showed no effects of glyphosate on GC at physiologically relevant test concentrations. Glyphosate had no effect on TH.

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 used and the tests were conducted with only one or 2 test concentrations of glyphosate.

Reliability criteria for *in vitro* toxicology studies

Publication: Perego *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)	N	Purity not reported. Source: Sigma Aldrich.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Test system clearly and completely described	Y	Bovine granulosa and theca cells.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y (partly)	0, 0.5, 5, or 0, 10, 300 µg/mL (1.77 mM), or 0, 5 µg/mL.
Cytotoxicity tests reported	Y?	Viability tested at only one concentration of glyphosate.
Biochemical methods described	Y?	Some could be more detailed.
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	Limited since max. 2 test concentrations.
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 tested was not sufficiently characterized, no positive controls were used and the tests were conducted with only one or 2 test concentrations of glyphosate.		

1. Information on the study

Data point:	CA 5.6
Report author	Perego M.C. *et al.*
Report year	2017
Report title	Evidence for direct effects of glyphosate on ovarian function: glyphosate influences steroidogenesis and proliferation of bovine granulosa but not theca cells *in vitro*
Document No	J. Appl. Toxicol. 2017; 37: 692–698
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restriction

2. Assessment and conclusion

Assessment and conclusion by applicant:

In this *in vitro* study, glyphosate had minimal effects on granulosa cells (GC). In the presence of FSH only, glyphosate had no effect on GC cell viability or on progesterone or estradiol production. In the presence of FSH and IGF1, glyphosate reduced GC proliferation without a dose-response at 0.5 and 5 μg ml^{-1} but not at lower test concentrations (0.01 and 0.3 μg ml^{-1}) and did not affect progesterone production or CYP19A1 and CYP11A1 mRNA expression; estradiol production was reduced at 5 μg ml^{-1} only (not at lower test concentrations). Without FSH or IGF1, 1.7 μg ml^{-1} of glyphosate slightly increased GC proliferation in response to serum (≤11%).

Glyphosate at 5 μg ml^{-1} had no effect on the theca cell (TC) proliferation or the production of progesterone or androstenedione.

Overall, with the exception of slight, non-dose-related alterations in GC proliferation under different test conditions, this study showed no effects of glyphosate on GC at physiologically relevant test concentrations. Glyphosate had no effect on TH.

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 used and the tests were conducted with only one or 2 test concentrations of glyphosate.

Reliability criteria for *in vitro* toxicology studies

Publication: Perego *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)	N	Purity not reported. Source: Sigma Aldrich.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Test system clearly and completely described	Y	Bovine granulosa and theca cells.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y (partly)	0, 0.5, 5, or 0, 10, 300 µg/mL (1.77 mM), or 0, 5 µg/mL.
Cytotoxicity tests reported	Y?	Viability tested at only one concentration of glyphosate.
Biochemical methods described	Y?	Some could be more detailed.
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	Limited since max. 2 test concentrations.
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 tested was not sufficiently characterized, no positive controls were used and the tests were conducted with only one or 2 test concentrations of glyphosate.		

1. Information on the study

Data point:	CA 5.6
Report author	Pham T. H. *et al.*
Report year	2019
Report title	Perinatal Exposure to Glyphosate and a Glyphosate-Based Herbicide Affect Spermatogenesis in Mice
Document No	Toxicological Sciences (2019) Vol. 169(1), 260–271
Guidelines followed in study	None
Deviations from current test guideline	NA
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

The effect of glyphosate exposure from the day of vaginal plug detection to 20 days post-partum via the drinking water at concentrations corresponding with 0.5, 5 and 50 mg/kg bw/day on male reproductive parameters in mice of 5, 20 and 35 days old and 8 months old was investigated. The parameters measured were the number of spermatogonia and expression of genes important to testicular function in 5-day old mice, testicular histopathology in 20-day old mice, relative weight of testes, epididymis and seminal vesicles, epididymal sperm count, serum testosterone levels, GATA1 positive cell count and ZBTB16 positive cell count in 35-day old mice, and relative weight of testes, epididymis and seminal vesicles and serum testosterone levels in 8-month old mice. No statistically significant change was found for the number of spermatogonia in 5-day old mice. The only genes of which the expression was statistically significantly changed in a dose-related fashion were Bcl2 and Kit. In 20-day old mice, sperm depleted seminiferous tubules were noted at 5 mg/kg bw but not at 0.5 and 50 mg/kg bw glyphosate. In 35-day old mice there was no statistically significant change in the relative weight of the epididymis and the seminal vesicles, epididymal sperm count and GATA1 positive cell count. No dose-effect relationship could be established for relative weight of testes, serum testosterone levels and ZBTB16 positive cell count. In 8-month old mice no statistically significant change could be observed for relative weight of epididymis and seminal vesicles and serum testosterone levels. No dose effect relationship could be established for the decrease in relative testes weight. From these data it can be concluded that there is no evidence that glyphosate dosed orally to mice up to 50 mg/kg bw/day during the perinatal period is an endocrine disruptor and has an adverse effect on testicular function and development. This has been corroborated by regulatory reproduction toxicology studies with rats at much higher dose levels.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test conditions were not clearly described and the number of animals tested per dose level is too limited.

Reliability criteria for *in vivo* toxicology studies

Publication: Pham *et al*., 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing	N	

guidelines		
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 ≥ 99.2%. Source: Sigma-Aldrich.
Only glyphosate acid or one of its salts is the tested substance	N	Also formulation was tested: Roundup 3 plus.
AMPA is the tested substance		
Study		
Test species clearly and completely described	Y?	
Test conditions clearly and completely described	Y?	Not completely described. Control and treated young prepubertal or adult mice were euthanized, and reproductive organs were dissected in 5, 20, 35 days old (d.o.), and in 8 months old (m.o.) mice. Only male mice were analysed.
Route and mode of administration described	Y	Oral via drinking water.
Dose levels reported	Y	0.5, 5, 50 mg/kg bw/day.
Number of animals used per dose level reported	Y?	5 animals derived from at least 3 to 4 different litters in each 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	Y?	Not always presented in tables.
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test conditions were not clearly described and the number of animals tested per dose level is too limited.		

1. Information on the study

Data point:	CA 5.5
Report author	Presutti R. et al.
Report year	2016
Report title	Pesticide exposures and the risk of multiple myeloma in men: An analysis of the North American Pooled Project
Document No	International Journal of Cancer (2016) Vol. 139, 1703–1714
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
Four population-based incident case-control studies (3 US studies and one Canadian study) pooled in the North American Pooled Project (NAPP) aimed to investigate the effects of pesticides and other agricultural exposures on the risk of lymphatic and hematopoietic cancers. The present analysis is restricted to a subset of three NAPP studies (Iowa, Nebraska and Canada) where multiple myeloma (MM) cases were recruited. Self-reported information on pesticide use, farming activities and demographic characteristics was collected and the odds ratios (OR) were calculated for "ever/never" exposure, years of exposure and cumulated lifetime days of exposure to glyphosate with and without exclusion of proxy respondents. The result is that no statistically significant increases in risk of multiple myeloma (MM) associated with self-reported exposure to glyphosate were observed.

This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because it concerns pooled case control studies which are subject to recall bias and in which confounding factors could not be ruled out.

Reliability criteria for epidemiology studies

Publication: Presutti *et al*., 2016	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	Y	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.i.		
Exposure to formulations with glyphosate combined with other a.i.		
Exposure to various formulations of pesticides	Y	
Study		
Study design – epidemiological method followed	Y	Pooled case control studies
Description of population investigated	Y	
Description of exposure circumstances	Y	May be subject to recall bias

Description of results	Y	
Have confounding factors been considered	N	Confounding factors cannot be ruled out
Statistical analysis	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because it concerns pooled case control studies which are subject to recall bias and in which confounding factors could not be ruled out.		

1. Information on the study

Data point:	CA 5.6
Report author	Ren X. *et al*.
Report year	2019
Report title	Effects of chronic glyphosate exposure to pregnant mice on hepatic lipid metabolism in offspring
Document No	Environmental pollution (2019) Vol. 254, pp. 112906.
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:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

The current study set out to examine any effects on lipid metabolism in fetuses and pups following prenatal exposure to glyphosate or the glyphosate formulation, Roundup™. Ten pregnant female rats per group were exposed from gestation day 1 through 19 to drinking water containing either 0.5% glyphosate, prepared using "pure" glyphosate (N-(phosphonomethyl) glycine), or 0.5% glyphosate using an appropriate dilution of Roundup™. A similar group of animals were given distilled water and served as the control group. Five females per group were terminated on gestation day 19 for examination of fetuses, while the remaining dams were allowed to litter and maintain their litters to postnatal day 21. Offspring (2/sex/litter where possible) were selected on postnatal days 7 and 21 for evaluation. Fetal and offspring evaluations included liver histology, serum biochemistry, liver lipid concentration and gene expression analysis of genes related to lipid metabolism in the liver.

The study is non-GLP and does not report the following information;

- Purity of test items
- Body weight and clinical signs for pregnant animals
- Clinical observations of offspring
- Achieved dose of glyphosate in treated animals in mg/kg bw/day.
- Measures to control inter animal and intergroup variability such as
    - Time of necropsy distributed equally across groups
    - Standardization of litter size on day 4 of lactation to mitigate variability caused by differences in liter size.
- More than a single dose level of glyphosate (0.5% solution); thus, preventing dose-response characterisation
- Liver weight of fetuses or offspring
- Normal physiological ranges for serum and liver biochemistry in this strain of rat at this laboratory
- Clear reporting of statistical evaluation and differences
- Thorough histological evaluations of the liver with incidence and severity of any recorded findings.

Although the authors concluded that there were treatment related effects on fetal and offspring body weight, there is no evidence from this study to suggest that glyphosate exposure has had any impact on fetal development or pup development postnatally. There was no effect on average birth weight of pups, the slight difference observed in the glyphosate treated group should be attributed to the slightly larger mean litter size observed (14.4 pups compared to 10.8 in the control group). Although figure 1 shows a reduction in mean pup weight in the glyphosate and Roundup™ treated

groups (male and females combined). Group mean body weight of pups by sex showed no statistical differences from control.

Given the small group size, the large inter animal variability observed, the lack of consistency between the same parameter across the sexes, timepoints or sampling matrices, and the deficiencies listed above, it not possible to clearly attribute any of the observed differences to glyphosate exposure. Therefore, the current study provides no evidence that glyphosate exposure causes lipid metabolism disruption in offspring following prenatal (in utero) exposure.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used is not sufficiently characterized, only one dose level was tested, there was large inter animal variability observed and too few animals per dose level were analysed.

Reliability criteria for *in vivo* toxicology studies

Publication: Ren *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Shanghai Ryon Biological Technology Co, Shanghai, China.
Only glyphosate acid or one of its salts is the tested substance	N	Also formulation was tested: Roundup from Sinochem Crop Protection Products, Shanghai, China.
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	?	Mice.
Test conditions clearly and completely described	?	
Route and mode of administration described	Y	Oral via drinking water.
Dose levels reported	Y	One dose level: 0.5% in drinking water.
Number of animals used per dose level reported	Y	10/dose.
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	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 is not sufficiently characterized, only one dose level was tested, there was large inter animal variability observed and too few animals per dose level were analysed.		

1. Information on the study

Data point:	CA 5.4
Report author	Roustan A. *et al.*
Report year	2014
Report title	Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation
Document No	Chemosphere (2014) Vol. 108, 93–100
Guidelines followed in study	OECD Test Guideline 487
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

The cytogenetic effect of two herbicides (glyphosate and atrazine), their metabolites (AMPA and DEA), and mixtures thereof was investigated in CHO-K1 cells in the *in vitro* micronucleus test. Only the results of glyphosate and AMPA tested alone are reported and discussed in the summary. Glyphosate and AMPA were tested with and without metabolic activation and with light irradiation. Also the potency of glyphosate and AMPA to produce ROS was investigated. The concentrations tested ranged from 5 to 100 µg/mL for glyphosate and from 0.00005 to 5 µg/mL for AMPA. No statistically significant increase in the incidence of bi-micronucleated cells (BMC) was observed with glyphosate at concentrations up to 100 ug/mL in the dark and without metabolic activation. However, a statistically significant and dose-related increase in BMC was noted from 10 µg/mL in the presence of metabolic activation. With light irradiation a statistically significant increase in BMC was noted for glyphosate at a concentration of 100 µg/mL. AMPA produced a statistically significant and dose-related increase in BMC from a concentration of 0.01 µg/mL in the dark and without metabolic activation. With metabolic activation a statistically significant increase in BMC was seen with AMPA from 1 µg/mL. With light irradiation the lowest test concentration of AMPA with a statistically significant increase in BMC was 0.0005 µg/mL. Only AMPA was found to produce an elevated oxidative effect, whereas the oxidative potency of glyphosate was very low. The results of glyphosate in the *in vitro* micronucleus test with metabolic activation reported in this study are surprising since glyphosate is essentially unmetabolized *in vitro* in the presence of a rat liver S9 homogenate. Moreover, these results are not corroborated by regulatory *in vivo* micronucleus tests in the mouse dosed up to more than 2,000 mg/kg bw.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate and AMPA tested were not sufficiently characterized and no positive and control historical data were reported. The *in vitro* micronucleus test carried out was in compliance with OECD TG 487.

Reliability criteria for *in vitro* toxicology studies

Publication: Roustan *et al*., 2014	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	Y	In vitro MN test compliant with OECD TG 487.
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 not reported. Source: Sigma-Aldrich Chemical Company, St Quentin-Fallavier, France.
Only glyphosate acid or one of its salts is the tested substance	N	Other pesticides (atrazine, desethyl atrazine (DEA)) were tested and mixtures thereof.
AMPA is the tested substance	Y	Tested alone and as mixtures with glyphosate, atrazine and DEA.
Test system clearly and completely described	Y	CHO-K1 cells.
Test conditions clearly and completely described	Y	CHO-K1 cell MN test.
Metabolic activation system clearly and completely described	Y	S9-mix.
Test concentrations in physiologically acceptable range (< 1 mM)	Y	From 5 to 100 µg/mL for glyphosate and from 0.00005 to 5 µg/mL for AMPA.
Cytotoxicity tests reported	Y	Cytokinesis Blocked Proliferation Index (CBPI) and incidence of bi-micronucleated cells (BMC).
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		
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 and AMPA tested were not sufficiently characterized and no positive and control historical data were reported. The *in vitro* micronucleus test carried out was in compliance with OECD TG 487.		

1. Information on the study

Data point:	CA 5.4
Report author	Santovino A. *et al.*
Report year	2018
Report title	In vitro evaluation of genomic damage induced by glyphosate on human lymphocytes
Document No	Environmental Science and Pollution Research (2018) 25:34693-34700s
Guidelines followed in study	Some compliance with OECD 473 and OECD 487
Deviations from current test guideline	Only continuous treatments in the absence of S9 were performed and these exceeded the 1.5 cell cycles recommended by both guidelines No historical control data are reported. Treatment commenced within 24 hours of PHA stimulation rather than 44-48 hours
Previous evaluation	No
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

This paper describes human lymphocyte chromosome aberration and micronucleus tests with glyphosate. Although broadly compliant with OECD 473 and 487 there are some critical deficiencies which will have adversely influenced the reliability of the findings. Treatment with glyphosate was initiated 24 hours after lymphocyte cultures were stimulated to divide, instead of the recommended 48 hours, consequently the cultures would not have been asynchronous. This could mean cells in some stages of the cell cycle may have been under-represented, whilst others over-represented. Exposure to glyphosate was continuous for 28 hours in the chromosome aberration assay or 48 hours in the micronucleus assay. In contrast OECD test guidelines recommend maximum exposure of 1.5 cell cycles, equivalent to approximately 24 hours for lymphocyte cultures. For both endpoints the paper does not confirm if the slides were coded prior to analysis. The positive control has been compared statistically to the glyphosate treated cultures rather than the solvent controls.

The authors consider that glyphosate induces tri-tetraradial aberrations (amongst other aberration types) but fails to comment that the frequency of these aberrations observed at a single glyphosate concentration is 3-fold lower than the frequency observed in the solvent control cultures. Furthermore, it is unusual that the only multi-aberrant metaphases observed were a small number of positive control metaphases and gaps did not appear to increase with treatment but chromatid and chromosome breaks did.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized and although the genotoxicity tests conducted were in general in accordance with the OECD test guidelines, significant deficiencies were noted.

Reliability criteria for *in vitro* toxicology studies

Publication: Santovito *et al.*, 2018	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing	Y	In accordance with

guidelines		OECD TG.
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-Aldrich, Saint 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	Lymphocytes.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.500, 0.100, 0.050, 0.025 and 0.0125 µg/mL.
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		
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 and although the genotoxicity tests conducted were in general in accordance with the OECD test guidelines, significant deficiencies were noted.		

1. Information on the study

Data point:	CA 5.9 Exposure study
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	International Journal of Environmental Research and Public Health (2019), Volume 16, Number 4, 562 p.
Guidelines followed in study	None.
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

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

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.i.		
Exposure to formulations with glyphosate combined with other a.i.		
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.		

1. Information on the study

Data point:	CA 5.5
Report author	Sorahan T.
Report year	2015
Report title	Multiple Myeloma and Glyphosate Use: A Re-Analysis of US Agricultural Health Study (AHS) Data
Document No	International Journal of Environmental Research and Public Health (2015) Vol. 12, 1548-1559
Guidelines followed in study	None
Deviations from current test guideline	NA
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

In this study the relative risk estimates for exposed and non-exposed applicators were calculated using Poisson regression and subjects with missing data were not excluded from the main analyses. When using the full dataset adjusted for age and gender the analysis produced a RR close to unity for ever-use of glyphosate. Additional adjustment for lifestyle factors and use of ten other pesticides had little effect. This study found no statistically significant trends of multiple myeloma risk with reported cumulative days of glyphosate use and unexceptional point estimates of risk for ever-use of glyphosate. This was irrespective of whether the analyses had adjustment for a few basic variables (age and gender) or adjustment for many other lifestyle factors or pesticide exposures, as long as data on all available pesticide applicators were used. The suspiciously elevated RRs reported previously arose from the use of restricted data sets that, probably by chance, turned out to be unrepresentative.

This publication concerns a secondary analysis of the data from the Agricultural Health Study (AHS) and is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the reliability criteria of a well conducted epidemiology study.

Reliability criteria for epidemiology studies

Publication: Sorahan T., 2015	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.		
Study completely described and conducted following scientifically acceptable standards	Y	Secondary analysis of the AHS data
Test substance		
Exposure to formulations with only glyphosate as a.i.		
Exposure to formulations with glyphosate combined with other a.i.		
Exposure to various formulations of pesticides	Y	
Study		

Publication: Sorahan T., 2015	**Criteria met? Y/N/?**	**Comments**
Study design – epidemiological method followed	Y	
Description of population investigated	Y	
Description of exposure circumstances	Y	
Description of results	Y	
Statistical analysis	Y	
Overall assessment		
Reliable withoiut restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication concerns a secondary analysis of the data from the agricultural health study and is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the reliability criteria of a well conducted epidemiology study.		

1. Information on the study

Data point	CA 5.9 Exposure study
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.	J Agric Food Chem (2016), Vol. 64, 1414−1421
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:	Yes/Reliable with restrictions

2. Assessment and conclusion

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 analyzed 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 analyzed 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 analyzed for glyphosate. In none of the samples analyzed 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 analyzed 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

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	Y	

Publication: Steinborn et al., 2016.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
scientifically acceptable standards		
Test substance		
Exposure to formulations with only glyphosate as a.i.		Self-reported exposure to pesticides.
Exposure to formulations with glyphosate combined with other a.i.		
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 analyzed 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 analyzed 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.		

1. Information on the study

Data point:	CA 5.4
Report author	Suárez-Larios K. *et al.*
Report year	2017
Report title	Screening of Pesticides with the Potential of Inducing DSB and Successive Recombinational Repair
Document No	Journal of Toxicology (2017), Article ID 3574840,
Guidelines followed in study	None
Deviations from current test guideline	NA
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
The objective of this study was to assess whether glyphosate and its metabolite AMPA produced DNA double strand breaks in human peripheral lymphocytes and whether proteins involved in DNA repair were induced. The results show that glyphosate, but not AMPA, increased the mean of the percent cells with more than 10 γ-H2AX foci, however, without a clear dose-effect relationship. Glyphosate was found to induce statistically significantly a protein involved in DNA repair, p-Ku80, at 5 μM without a dose-effect relationship (when measured as median OD).

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because glyphosate as a test chemical was not sufficiently characterized and the effect found on an indicator of DNA double strand breaks was not concentration related and occurred at concentrations that were much lower than the systemic concentrations (approx. 300 μM) of regulatory *in vivo* MN tests at 2000 mg/kg bw which were negative.

Reliability criteria for *in vitro* toxicology studies

Publication: Suarez-Larios *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?	No guideline study for genotoxicity
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Only source reported: Sigma-Aldrich Mexico.
Only glyphosate acid or one of its salts is the tested substance	Y	
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	N	Not applied

Test concentrations in physiologically acceptable range (< 1 mM)	Y	Up to 50 µM
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	SS increase at 2 concentrations but no dose effect relationship for γ-H2AX foci. SS increase of P-Ku80 at 5 µM but no dose-effect relationship in treated cultures (measured as median OD)
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	Results of this study are not corroborated by in vivo MN studies with much higher blood concentrations (approx. 300µM).
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because glyphosate as a test chemical was not sufficiently characterized and the effect found on an indicator of DNA double strand breaks was not concentration related and occurred at concentrations that were much lower than the systemic concentrations (approx. 300 µM) of regulatory *in vivo* MN tests at 2000 mg/kg bw which were negative.		

1. Information on the study

Data point:	CA 5.3
Report author	Tang J. *et al.*
Report year	2017
Report title	Ion imbalance Is Involved in the Mechanisms of Liver Oxidative Damage in Rats Exposed to Glyphosate
Document No	Frontiers in Physiology (2017) Vol. 8, Article 1083
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

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

This publication is considered relevant but reliable with restrictions because the test material was not sufficiently characterized, the number of animals used for this study duration is not sufficient, the results were not always accurately reported and are not corroborated by regulatory toxicology studies of similar test durations and dose ranges.

Reliability criteria for *in vivo* toxicology studies

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

Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	
Dose levels reported	Y	
Number of animals used per dose level reported	Y	
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	Sometimes inaccurate reporting of data in tables. Results not concordant with short term regulatory toxicology studies
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant but reliable with restrictions because the test material was not sufficiently characterized, the number of animals used for this study duration is not sufficient, the results were not always accurately reported and are not corroborated by regulatory toxicology studies of similar test durations and dose ranges.		

1. Information on the study

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	Food and Chemical Toxicology (2013) Vol. 59, 129–136
Guidelines followed in study	None
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

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

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.		

1. Information on the study

Data point:	CA 5.4
Report author	Townsend M. *et al.*
Report year	2017
Report title	Evaluation of various glyphosate concentrations on DNA damage in human Raji cells and its impact on cytotoxicity
Document No	Regulatory Toxicology and Pharmacology (2017) Vol. 85 79-85
Guidelines followed in study	None
Deviations from current test guideline	NA
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

The purpose of this study was to investigate the concentration and time dependent DNA damaging potential of glyphosate in Burkitt's B Cell Lymphoma (Raji) cells using the comet assay and MTT viability assay. The cells were exposed to glyphosate concentrations ranging from 0.1 µM to 15 mM and resulting DNA damage and loss of cell viability were measured after various lengths of exposure. DNA damage could only be observed at 1mM and higher which are concentrations that cannot be attained in vivo. The DNA damage seen at 1 and 5 mM reached its maximum between 60 and 80 minutes of incubation which returned to control values thereafter. To reach 1 mM of systemic concentration *in vivo* experimental animals have to be treated orally with glyphosate at dose levels that are much higher than those used in long term carcinogenicity studies which showed no carcinogenic effect of glyphosate.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with most of the reliability criteria for *in vitro* testing although no historical control data were reported. The significance for the risk assessment of glyphosate is limited because DNA damage has only been demonstrated at concentrations of glyphosate that cannot be attained in *in vivo* test systems.

Reliability criteria for *in vitro* toxicology studies

Publication: Townsend *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 95%. Source: not reported.
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	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y/N	0.1 µM to 15 mM for alkaline comet assay
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	Human cell exposure to glyphosate has minimal cytotoxicity and DNA damage at concentrations at or below 100 µM. Only effects found beyond 1 mM.
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 most of the reliability criteria for in vitro testing although no historical control data were reported.		

1. Information on the study

Data point:	CA 5.9 Exposure study
Report author	Trasande L. *et al.*
Report year	2020
Report title	Glyphosate exposures and kidney injury biomarkers in infants and young children
Document No	Environ Pollution (2020), 256, 113334
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Yes, all research was performed in accordance with relevant guidelines/regulations.
Acceptability/Reliability:	Yes/Reliable without restriction

2. Assessment and conclusion

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

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.i.	Y	
Exposure to formulations with glyphosate combined with other a.i.	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.

Publication: Trasande et al., 2020.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
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.		

1. Information on the study

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	Toxicology in Vitro (2018) Vol. 52, 14–22
Guidelines followed in study	None
Deviations from current test guideline	NA
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

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

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 acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	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.		

1. Information on the study

Data point:	CA 5.5
Report author	Wang L. *et al.*
Report year	2019
Report title	Glyphosate induces benign monoclonal gammopathy and promotes multiple myeloma progression in mice
Document No	Hematol Oncol 12, 70 (2019)
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:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

The objective of this study was to investigate the pathogenic role of glyphosate in multiple myeloma using Vk*MYC mice. The study did demonstrate the ability of glyphosate to impact measured parameters in the tested models. However, this study is not appropriate for human health risk assessment. The number or animals per group was below the recommended number for guideline toxicity studies and to perform sufficient statistical analysis. Only one dose level was used in the chronic study. It was not possible to correlate effects with a glyphosate dose-response as the water consumption of animals was not provided and is therefore impossible to calculate a dose on a mg/kg bw basis for risk assessment.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, only one dose was considered for the chronic study and the number of animals used per group was either too low (acute study) or not reported (chronic study).

Reliability criteria for *in vivo* toxicology studies

Publication: Wang *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance		
AMPA is the tested substance		
Study		

Test species clearly and completely described	Y	Wild-type (WT) C57Bl/6 mice and Vk*MYC mice.
Test conditions clearly and completely described		
Route and mode of administration described	Y	Oral via drinking water.
Dose levels reported	Y	For the chronic study: 1 g/L in drinking water for 72 weeks. For the acute study: 1, 5, 10, 30 g/L for 7 days.
Number of animals used per dose level reported	Y	5/group for the acute study, no/group not reported for the chronic study.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	N	Not possible. Only one dose level used.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, only one dose was considered for the chronic study and the number of animals used per group was either too low (acute study) or not reported (chronic study).		

1. Information on the study

Data point:	CA 5.5
Report author	Wozniak E. *et al.*
Report year	2020
Report title	Glyphosate affects methylation in the promoter regions of selected tumor supressors as well as expression of major cell cycle and apoptosis drivers in PBMCs (in vitro study)
Document No	Toxicology in Vitro 63 (2020)
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:	Yes/Reliable without restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

The objective was to assess epigenetic mechanisms of action of glyphosate in human PBMCs, which has been poorly studied in cellular models including blood cells. The study was conducted using an *in vitro* test system. The ability of glyphosate to impact the measured parameters was demonstrated (global DNA methylation of PBMCs, methylation in the promoter regions of selected tumor suppressors (P21 and TP53), and expression of major cell cycle and apoptosis drivers (P16, TP53, BCl2, CCND1 and P21). However, a positive control was not used, and a clear dose-response was not established for all of the measured parameters. Additionally, the measured effects in vitro are not clearly linked to an adverse outcome in vivo. While it is stated that the concentrations used are comparable to environmental exposure, external exposure was not linked to a corresponding internal concentration. Therefore, it is not possible to calculate a dose for risk assessment purposes. The study is useful for supplemental information on *in vitro* effects resulting from glyphosate exposure, but, is not appropriate for derivation of an endpoint in human health risk assessment.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because the assays conducted comply in general with the quality criteria for *in vitro* testing.

Reliability criteria for *in vitro* toxicology studies

Publication: Wozniak *et al*., 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 95%. Source: Sigma-Aldrich, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	PBMC.

Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.5 to 100 µM
Cytotoxicity tests reported	Y	Ref. to earlier paper.
Positive and negative controls	Y	Methylated control DNA as positive control.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because the assays conducted comply in general with the quality criteria for in vitro testing.		

1. Information on the study

Data point:	CA 5.6
Report author	Zhang, J-W. *et al.*
Report year	2019
Report title	The toxic effects and possible mechanisms of glyphosate on mouse oocytes
Document No	Chemosphere 237 (2019) 124435
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:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

In vitro intracellular changes in Kunming mice oocytes were evaluated after being cultured in medium supplemented with 500 μM glyphosate. Findings included: decreased germinal vesicle breakdown, decreased first polar body extrusion, increased mRNA expression of anti-oxidant enzyme related genes, abnormal spindle morphology, increased DNA double strand breaks, aggregated mitochondria, decreased mitochondrial membrane potential, increased protein expression of apoptosis factors, increased mRNA expression of apoptosis related genes and decreased autophagy-related genes.

No dose-response could be determined seeing as only one concentration was tested, far in excess of that considered biologically relevant. Whilst some evaluations were conducted on oocytes harvested from a wider data set of 24 mice (protein expression levels of apoptosis factors by Western blot analysis), a number of the assessments were conducted on oocytes from just 12 mice (mRNA expression of oxidative stress-related, apoptosis-related and autophagy-related genes) or 6 mice (mitochondrial staining, measurement of mitochondrial membrane potential). This narrow source of oocytes limits the robustness of certain conclusions. Furthermore, there are insufficient details reported in the methods to establish whether mice were of the same age before oocyte harvesting or the purity of the glyphosate tested.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the poor characterization of the glyphosate tested, no cytotoxicity testing, the lack of a positive control and insufficient dose-response characterization at biologically relevant doses.

Reliability criteria for *in vitro* toxicology studies

Publication: Zhang JW *et al*., 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		

Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity and source not reported. Chemical analysis was performed but the results were not clear.
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	50, 100, 200, 500 µM. Most of the assays were carried out at 500 µM which is a concentration that cannot be reached systemically in the rat at 2000 mg/kg bw after oral intake.
Cytotoxicity tests reported	N	
Positive and negative controls	N	No positive control 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	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the poor characterisation of the glyphosate tested, no cytotoxicity testing and the lack of a positive control.		