検索期間：2010～2019年

区分ａに分類された文献とその理由

ヒトに対する毒性

1. Information on the study

Data point:	CA 5.4
Report author	Adler-Flindt S. *et al.*
Report year	2019
Report title	Comparative cytotoxicity of plant protection products and their active ingredients
Document No	Toxicology in Vitro (2019) Vol. 54, 354-366
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

It was the intention of this study to evaluate the GHS classification of pesticide formulations for acute toxicity based on calculated LD_{50} values using the CLP calculation method (CM). Because of the considerable inaccuracy of this method the *in vitro* cytotoxicity of 10 pesticide formulations was compared against that of the active ingredient using mouse (3T3) and human (hFF) fibroblasts. In this exercise the IC_{50} for glyphosate isopropylamine salt was found to be 954.8 ± 117.1 µg/mL for 3T3 cells and 1211 ± 885.7 µg/mL for hFF cells and the IC_{50} for MON 52276 was 313.2 ± 29.3 µg/mL for 3T3 cells and 361.6 ± 612 µg/mL for hFF cells. The difference in cytotoxicity (expressed as the AUC of the % viability vs concentration curve) between glyphosate and MON 52276 could be regarded as minor.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate isopropylamine salt used was not sufficiently characterized and the standard deviation of the IC_{50} of glyphosate (1211 ± 885.7 ug/mL) and MON 52276 (361.6 ± 612 µg/mL) for human fibroblasts is too large.

Reliability criteria for *in vitro* toxicology studies

Publication: Adler-Flindt *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	Glyphosate isopropylamine salt, purity not reported. Source: Monsanto Agrar Deutschland GmbH, Düsseldorf, Germany.
Only glyphosate acid or one of its salts is the tested	N	MON 52276

substance		(RoundUP LB Plus, 360 g/L A.I.). Source : Monsanto Agrar Deutschland GmbH, Düsseldorf, Germany. Other pesticides and their formulations were tested as well.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Mouse (3T3) and human (hFF) fibroblasts.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y (for local contact)	Test concentrations up to 1000 µg/mL which is beyond the systemic physiological range but not when applied dermally.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	Saccharin was used as the negative control and 5-FU as the 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	IC_{50} were calculated.
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 isopropylamine salt used was not sufficiently characterized and the standard deviation of the IC_{50} of glyphosate (1211 ± 885.7 ug/mL) and MON 52276 (361.6 ± 612 µg/mL) for human fibroblasts is too large.		

1. Information on the study

Data point:	CA 5.5
Report author	Andreotti, G. et al.
Report year	2018
Report title	Glyphosate Use and Cancer Incidence in the Agricultural Health Study
Document No	Journal of the National Cancer Institute (2018), Vol. 110, No. 5, pp. 509-516
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

The AHS is an ongoing prospective cohort study of glyphosate and other pesticides. It was initiated in 1993 and has been ongoing for more than 25 years. Researchers from the US National Cancer Institute and other government agencies initiated the AHS as a prospective cohort study to eliminate the possibility of case-recall bias – an intractable potential bias in case control studies that rely on self-reported exposure information. Crump (Risk Analysis DOI:10.1111/risa.13440) has recently illustrated that the results from the glyphosate case-control studies align closely with what would be expected from case recall bias.

In addition to obviating concerns about case-recall bias, the Andreotti et al. publication is noteworthy on several counts. First, the frequency of glyphosate use by participants (median = 48 days, IQR 20 to 166 days) vastly exceeds that in the glyphosate case-control studies. In those studies the most frequent days of use category is > 10 days (Eriksson M, et al. Int J Cancer. 2008; 123:1657-1663), while most of the case control studies' primary analyses were based on 1 day or more of use in a lifetime. Second, the participants in the AHS were licensed pesticide applicators who were considered by the authors to be very capable to report pesticide use accurately compared with other study populations. Third, the analyses by Andreotti et al. controlled for a multitude of personal factors and for other pesticides in addition to incorporating a wide range of sensitivity and lagged analyses (allowing for up to 20+ years induction-latency). No other study has evaluated the relationship between glyphosate use and cancers as extensively. The AHS is, by far, the most informative and relevant study epidemiologic study for glyphosate to date.

Accordingly, given the AHS results for NHL among those with extensive glyphosate use (n = 111 exposed cases, RR = 0.9, 95% CI 0.6 – 1.2), it is unlikely that the positive associations for glyphosate and NHL in some case control studies are valid. As follow-up of the AHS cohort continues, it remains to be seen whether subsequent results will identify relationships between individual cancers and glyphosate use that are relevant for risk evaluations.

Reliability Criteria: Epidemiology studies

Publication: Andreotti G. *et al.,* 2018	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	Yes	
Appropriate study population to address potential glyphosate-related health outcomes	Yes	Most appropriate population studied to date. Highest frequency of glyphosate use by far.
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	Yes	
Comparable participation by groups being compared	Yes	
Information provided by proxy respondents	No	
Adequate statistical analysis	Yes	Very comprehensive
Adequate consideration of personal confounding factors	Yes	Very comprehensive
Adequate consideration of potentially confounding exposures	Yes	Very comprehensive
Overall assessment		
Reliable without restrictions	Yes	Most reliable epidemiology study for glyphosate users versus non-users.
Reliable with restrictions	Yes	Certain analyses are limited: dose is not known, only frequency of use. So, “dose response” analyses must be interpreted cautiously.
Not reliable	No	

1. Information on the study

Data point:	CA 5.5
Report author	Biserni M. *et al.*
Report year	2019
Report title	Quizalofop-p-ethyl induces adipogenesis in 3T3-L1 Adipocytes
Document No	Toxicological Sciences (2019), Vol. 170(2), 452–461
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
In this study glyphosate, among other pesticide active ingredients, was investigated for its effect on lipid accumulation in differentiated adipocytes *in vitro* at concentrations ranging from 0.1 to 1000 µM. The results indicated that at the concentrations tested glyphosate scored negative for lipid accumulation. This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions.

Reliability criteria for *in vitro* toxicology studies

Publication: Biserni *et al*., 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of ≥ 96%. Source: Sigma-Aldrich, Gillingham, UK
Only glyphosate acid or one of its salts is the tested substance	N	Other pesticide active ingredients were tested as well.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Concentration range *in vitro* from 0.1 to 1000 µM.
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	Glyphosate was not tested in all tests described.
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	No effect of glyphosate over the entire concentration range tested.
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions.		

1. Information on the study

Data point:	CA 5.7
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	Toxicological sciences: (2013) Vol. 133, No. 2, pp. 289-97.
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 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 relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate isopropylamine salt used was not sufficiently characterized and the standard deviation of the IC_{50} of glyphosate (1211 ± 885.7 ug/mL) and MON 52276 (361.6 ± 612 µg/mL) for human fibroblasts is too large.		

1. Information on the study

Data point:	CA 5.9 Exposure study
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	International Journal of Hygiene and Environmental Health (2017) Vol. 220, 1064–1073
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:

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

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

Publication: Connolly *et al*., 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
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.		

1. Information on the study

Data point:	CA 5.9 Exposure study
Report author	Connolly A. *et al.*
Report year	2018
Report title	Characterising glyphosate exposures among amenity horticulturists using multiple spot urine samples
Document No	International journal of Hygiene and Environmental Health (2018) Vol. 221, 1012-1022
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

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 analyzed 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

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.i.	Y	
Exposure to formulations with glyphosate combined with other a.i.	N	
Exposure to various formulations of pesticides	N	

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.		

1. Information on the study

Data point:	CA 5.9 Exposure study
Report author	Connoly A. *et al.*
Report year	2018
Report title	Glyphosate in Irish adults – A pilot study in 2017
Document No	Environmental Research 165 (2018) 235-236
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 without restrictions

2. Assessment and conclusion

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

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

1. Information on the study

Data point:	CA 5.9 Exposure study
Report author	Connolly A. *et al.*
Report year	2019
Report title	Evaluating glyphosate exposure routes and their contribution to total body burden: a study among amenity horticulturalists
Document No	Annals of Work Exposures and Health, 2019, 63 (2), 133–147
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 without restrictions

2. Assessment and conclusion

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

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.i.	Y	
Exposure to formulations with glyphosate combined with other a.i.	Y	
Exposure to various formulations of pesticides	Y	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	

Reliability criteria of exposure studies

Publication: Connolly *et al.*, 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
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.		

1. Information on the study

Data point:	CA 5.9 Exposure study
Report author	Connolly A. *et al.*
Report year	2019
Report title	Exploring the half-life of glyphosate in human urine samples
Document No	International Journal of Hygiene and Environmental Health (2019) Vol. 222, 205-210
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

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

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

Publication: Connolly *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
testing guidelines/practices.		
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.i.	Y	
Exposure to formulations with glyphosate combined with other a.i.	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.		

1. Information on the study

Data point:	CA 5.9 Exposure study
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	International Journal of Hygiene and Environmental Health 220 (2017) 8-16
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 without restrictions

2. Assessment and conclusion

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

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.i.	NA	Exposure to glyphosate and AMPA mainly through the diet.
Exposure to formulations with glyphosate combined with other a.i.	NA	
Exposure to various formulations of pesticides	NA	
Study		

Reliability criteria of exposure studies

Publication: Conrad *et al.* 2017	Criteria met? Y/N/?	Comments
Guideline-specific		
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.		

1. Information on the study

Data point:	CA 5.5
Report author	Crump K.
Report year	2019
Report title	The potential effects of recall bias and selection bias on the epidemiological evidence for the carcinogenicity
Document No	Risk Anal. (2020); 40(4):696-704
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable

2. Assessment and conclusion

Assessment and conclusion by applicant:

It is well known that recall bias is a potentially important bias in cancer case control studies where study participants are asked to recall their past exposures. In an ideal study, information about exposures for cases and controls would be collected under exactly the same circumstances. However, circumstances are quite different for cases and controls. Cancer cases have suffered a grievous illness and it is only natural for them to be deeply introspective about what might have caused their cancers. Controls have no such motivation that would augment their recall (or reporting). So, the concern expressed in many textbooks is that recall bias tends to produce false positive results. The purpose of this analysis by Crump was to evaluate the evidence for recall bias in the overall pattern of results in five case control studies and two cohort studies that comprise the main part of the glyphosate-NHL literature.

In evaluating the case control studies, Crump reasoned that the percentage of odds ratios > 1 for non-glyphosate exposures should be approximately 50% if recall bias was not operative and those exposures did not cause NHL. Yet, it turned out that the percentages of ORs >1 for non-glyphosate exposures were 90% for Hardell et al. (2002), 90% for Erikson et al. (2008), 93% for McDuffie et al. (2001), 76% for Orsi et al. (2009), and 53% for DeRoos et al. (2003). These extreme departures from 50% for 4 of the 5 case control studies is consistent with recall bias, perhaps augmented by a type of selection bias in the analyses by Hardell et al. (2002) and Eriksson et al. (2008). In contrast, in the most recent publication from the Agricultural Health Study (Andreotti et al. 2018), only 48% of the relative risks (RR) calculated were >1 – a percentage in the range expected with a true probability of 50%. While the evaluation of Andreotti et al. (2018) concerned glyphosate and other cancer sites and not other exposures and NHL, the principle is the same: under the null hypothesis the proportion of ORs or RRs > 1 should be roughly 50% absent bias.

We agree with Crump's conclusion that the 4 case-control studies with a high proportion of ORs > 1 are "contaminated" by statistical bias and are not reliable as evidence of a relationship between glyphosate and NHL. Of course, there are also other types of bias that may contribute to the high proportion of positive ORs (e.g., lack of control for confounding, lower participation for controls than cases (traditional selection bias), proxy respondents, etc.) (see Acquavella et al. 2016). Nonetheless, Crump's point is well taken that ORs for glyphosate in 4 of the 5 case control studies should be interpreted as unreliable because the vast majority of ORs for other exposures are >1.

Reliability Criteria: Epidemiology studies

Publication: Crump K., 2019	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	Yes	For a methodologic evaluation of recall bias in existing studies
Appropriate study population to address potential glyphosate-related health outcomes	Not applicable	
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Not applicable	
Adequate description of exposure circumstances	Not applicable	
Comparable participation by groups being compared	Not applicable	
Information provided by proxy respondents	Not applicable	
Adequate statistical analysis	Yes	To illustrate bias
Adequate consideration of personal confounding factors	Not applicable	
Adequate consideration of potentially confounding exposures	Not applicable	
Overall assessment		
Reliable without restrictions	Yes	As methodologic work. Clearly illustrates recall bias in the glyphosate case control studies.
Reliable with restrictions	No	
Not reliable	No	

1. Information on the study

Data point:	NA Zebra fish cell line
Report author	da Silva N. D. G. *et al.*
Report year	2020
Report title	Interference of goethite in the effects of glyphosate and Roundup® on ZFL cell line
Document No	Toxicology in Vitro 65 (2020) 104755
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:

It was shown in this *in vitro* study that goethite NPs and Roundup® presented cytotoxic and genotoxic effects in ZFL cells and, when co-exposed, produced a synergistic effect. Glyphosate did not promote cytotoxic, biochemical, or genotoxic damage to ZFL cells and, in association, the toxic effects produced by isolated goethite NPs were suppressed by glyphosate. It is concluded therefore that these findings indicate that the presence of other compounds in the formulated product may be responsible for the aquatic organism toxicity of this herbicide when compared to the active ingredient glyphosate.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test system and glyphosate were not sufficiently characterized and only one concentration of glyphosate was used for testing, preventing any characterisation of dose-response.

Reliability criteria for *in vitro* toxicology studies

Publication: Da Silva *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)	N	Purity of glyphosate is not reported, only source (Milenia Agrociencias S/ A)
Only glyphosate acid or one of its salts is the tested substance	N	Also formulation was tested
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	N	
Test conditions clearly and completely described	Y	

Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Only one concentration of glyphosate tested: 3.6 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	N	Only one concentration of glyphosate tested
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 system and glyphosate were not sufficiently characterized and only one concentration of glyphosate was used for testing.		

1. Information on the study

Data point:	CA 5.6
Report author	Dai P. *et al.*
Report year	2016
Report title	Effect of glyphosate on reproductive organs in male rat
Document No	Acta Histochemica (2016) Vol. 118, 519–526
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 potential toxicity of glyphosate to the male reproductive system of the rat has been investigated after oral treatment with glyphosate for 5 weeks at dose levels up to 500 mg/kg bw. The endpoints studied were body weight, food intake, daily weight gain, absolute and relative reproductive organ weight, serum hormone levels, oxidative stress parameters, testicular histopathology and expression of AR in testis. The effects found were a significant decrease in absolute (but not relative) weight of the seminal vesicle gland and coagulating gland and a decrease in sperm count at the highest dose tested.

This publication is considered relevant but reliable with restrictions because there are deviations from regulatory guidelines for reproductive toxicology studies and the reproductive effects seen are not corroborated by the results from regulatory studies at similar dose levels.

Reliability criteria for *in vivo* toxicology studies

Publication: Dai *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?	Incomplete study
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 90% as isopropylamine salt. Source Shanghai Ryon Biological Technology Co. Ltd., China.
Only glyphosate acid or one of its salts is the tested substance	Y	Isopropylamine salt
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	8 males per 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?	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	Results are not concordant with outcome of regulatory reproduction toxicology studies
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant but reliable with restrictions because there are deviations from regulatory guidelines for reproductive toxicology studies and the reproductive effects seen are not corroborated by the results from regulatory studies at similar dose levels.		

1. Information on the study

Data point:	CA 5.4
Report author	De Almeida L. K. S. *et al.*
Report year	2018
Report title	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
Document No	3 Biotech (2018) Vol. 8(10), 438 (1-15)
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 cytotoxicity of glyphosate has been investigated in whole blood, in hormone independent (MDA-MB-231) and in hormone responsive (MCF7) cell lines and in a an endometrial cancer cell line (HEC1A). The capacity of glyphosate to produce DNA damage was investigated in MCF7, MDA-MB-231 and HEC1A cells in the Comet assay. Glyphosate was found to reduce cell viability in whole blood at the intermediate concentrations (10-250 µg/mL) but not at the highest concentration tested (500 µg/L). A concentration related reduction in cell viability was seen with glyphosate in HEC1A cells but not in the two other cell lines. When glyphosate was tested at 500 and 1000 µg/mL an increase in tail length and tail moment was observed in HEC1A and MDA-MB-231cells but not in the hormone responsive breast cancer cell line MCF7. The *in vitro* concentrations of glyphosate at which DNA damage was observed were 500 and 1,000 µg/mL which are systemic concentrations that cannot be reached in *in vivo* toxicology studies.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the Comet assay was only conducted at concentrations that are physiologically not feasible in *in vivo* toxicology studies (> 1mM).

Reliability criteria for *in vitro* toxicology studies

Publication: De Almeida 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	?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 99.5%. Source: Supelco Analytical USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also glyphosate-based formulations were tested.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Whole blood from volunteers, breast cancer cells (MCF7 and MDA-MB-231) and endometrial cancer cells (HEC1A).
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	N	For cytotoxicity testing glyphosate concentrations from 0.1 to 500 µg/mL were used. For comet testing only glyphosate concentrations of 500 and 1000 µg/mL were used (> 1 mM).
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	Was studied but not established.
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 Comet assay was only conducted at concentrations that are physiologically not feasible in *in vivo* toxicology studies (> 1mM).		

1. Information on the study

Data point:	CA 5.5
Report author	Duforestel M. *et al.*
Report year	2019
Report title	Glyphosate primes mammary cells for tumorigenesis by reprogramming the epigenome in a TET3-dependent manner
Document No	Frontiers in genetics, (2019) Vol. 10, pp. 885.
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 DNA hypomethylation in MCF10A cells, tumorigenic response for MCF10A Cells in a two-factor hit model, prevention of tumor formation in glyphosate-challenged cells, and TET3-Mediated Gene Demethylation following glyphosate exposure. This study was conducted in vitro using only one level of glyphosate. Glyphosate was not correlated to environmental exposures. In the in vivo portion of the study, a sufficient number of animals were not used to determine a carcinogenic response for statistical analysis. While this study is acceptable as supplemental information on the in vitro effects of glyphosate, it is not appropriate for endpoint derivation in human health risk assessment.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was insufficiently characterized and only one and extremely low concentration of glyphosate was used.

Reliability criteria for *in vitro* toxicology studies

Publication: Duforestel *et al*., 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity was not reported, source: Santa-Cruz, France.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Non-neoplastic breast epithelial MCF10A cells.
Test conditions clearly and completely described	Y	

Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	One test concentration at 10^{-11}M, 10^{-5} µM (extremely low concentration) applied every 3 to 4 days over 21 days.
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Not possible with one concentration
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was insufficiently characterized and only one and extremely low concentration of glyphosate was used.		

1. Information on the study

Data point:	CA 5.6
Report author	Forgacs A. L. *et al.*
Report year	2012
Report title	BLTK1 Murine Leydig Cells: A Novel Steroidogenic Model for Evaluating the Effects of Reproductive and Developmental Toxicants
Document No	Toxicological Sciences (2012) Vol.127(2), 391–402
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:
In this study, recombinant human chorionic gonadotropin (rhCG) and forskolin (FSK) were used as positive controls for the induction of steroidogenesis, as measured by increases in progesterone, testosterone and 17β-estradiol levels in culture media. Murine BLTK1 Leydig cells were investigated as a novel model for evaluating the effects of chemicals on steroidogenesis. The results demonstrated that BLTK1 cells can be used to screen substances that alter intracellular cAMP, steroidogenic gene expression, and sex steroid levels. When tested in this system glyphosate was not found to induce testosterone production or alter rhCG induction of testosterone.

This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because the test substance was not characterized and the results of only one concentration level were reported.

Reliability criteria for *in vitro* toxicology studies

Publication: Forgacs *et al*., 2012	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.
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)		

Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Only one concentration was tested (300 μM) Glyphosate, did not induce or alter rhCG induction of T. Glyphosate also had no effect on T levels in BLTK1 cells
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 the test substance was not characterised and only one concentration level was tested.		

1. Information on the study

Data point:	CA 5.8.2
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	Frontiers in Public Health (2018) Vol. 6, 103
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

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

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.		

1. Information on the study

Data point:	CA 5.3
Report author	Gao H. *et al.*
Report year	2019
Report title	Activation of the N-methyl-D-aspartate receptor is involved in glyphosate-induced renal proximal tubule cell apoptosis
Document No	Journal of Applied Toxicology (2019) Vol. 39(8), 1096-1107
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

In this study the effect of glyphosate on human proximal tubular epithelial cells was studied *in vitro* and on kidney *in vivo*. Tubular epithelial cells (HK-2) *in vitro* were exposed to glyphosate at concentrations ranging from 20 to 100 μM whereas mice were orally treated with glyphosate at 400 mg/kg bw/day for 28 days. The endpoints investigated for the *in vitro* study were cell viability, apoptosis, oxidative stress, intracellular Ca^{2+}, expression of the N-methyl-D-aspartate (NMDA) receptor and expression of proteins involved in apoptosis. The endpoints explored in the *in vivo* study in mice were kidney pathology biomarkers, oxidative stress in kidney tissue, kidney histopathology, NMDA receptor immunochemistry and apoptosis in kidney tissue. The results were that glyphosate was found to reduce cell viability, increase the incidence of apoptotic cells with an increase in the expression of apoptosis-related proteins, increase of oxidative stress in a concentration-related manner, increase of the expression of the NMDA receptor and increase Ca^{2+} influx. Kidney histopathology in mice treated with glyphosate at 400 mg/kg bw/day for 28 days revealed the exfoliation of renal tubular cells. It is postulated by the authors that glyphosate could affect renal tubule epithelial cells via the NMDAR1/$[Ca^{2+}]_i$/ROS pathway.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because some of the biochemical methods are not completely described, only one dose was used in the *in vivo* study and the pathology results from the *in vivo* study are not corroborated by regulatory 90-day repeated dose toxicity studies where no renal effects were seen in rats dosed up to more than 4,000 mg/kg bw/day and mice dosed up to more than 7,000 mg/kg bw/day.

Reliability criteria for *in vitro* toxicology studies

Publication: Gao *et al.*, 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 96% as glyphosate monoisopropylamine

		salt. Source: Millipore Sigma, St. Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Concentration range *in vitro* from 20 to 100 µM. Only one dose (400 mg/kg bw/day) was used in the oral toxicity study in mice.
Cytotoxicity tests reported	Y	
Biochemical methods described	Y?	Some could be better documented.
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	*In vitro* but not *in vivo* (only one dose used).
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because some of the biochemical methods are not completely described, only one dose was used in the *in vivo* study and the pathology results from the *in vivo* study are not corroborated by regulatory 90-day repeated dose toxicity studies where no renal effects were seen in rats dosed up to more than 4,000 mg/kg bw/day and mice dosed up to more than 7,000 mg/kg bw/day.		

1. Information on the study

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
Document No	Animal Reproduction Science (2018) Vol. 195, 185–196
Guidelines followed in study	None
Deviations from current test guideline	NA
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

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-effect 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-effect 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

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.		

1. Information on the study

Data point:	CA 5.6
Report author	Gorga A. *et al.*
Report year	2020
Report title	In vitro effects of glyphosate and Roundup on Sertoli cell physiology
Document No	Toxicology in Vitro 62 (2020) 104682
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:

This *in vitro* investigation showed that exposure to G and R at 100 ppm alters Sertoli cell junction barrier permeability, measured by decreased TER, and also decreased testosterone-stimulated TER. This study also showed that, at least in part, the loss of location of claudin11 at the interface between neighboring Sertoli cells might be responsible for the disassembly of the barrier. G or R did not modify androgen receptor mRNA or protein levels, nor did G modify P-p38-MAPK and P-ERK1/2 signalling pathways involved with BTB integrity at any doses tested, or affect the expression of intercellular junction proteins (claudin11, occludin and ZO-1). However, G and R induced redistribution of claudin11 at the zone of contact between cells. Neither G nor R modified lactate production, glucose uptak, GLUT1, FA oxidation, or *FAT/CD36* and *CPT1* expression in SC, thus indicating no effect of G or R on SC nutritional function or metabolism.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterized, there was no positive control and most of the endpoints were tested at only 2 concentrations preventing any dose response evaluations, with the highest concentration exceeding that which is physiologically relevant.

Reliability criteria for *in vitro* toxicology studies

Publication: Gorga *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)	N	Purity not reported. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also formulation was tested: Roundup Full II (Monsanto, Argentina)
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		
Test concentrations in physiologically acceptable range (< 1 mM)	Y	3 test concentrations used of which the highest was > 1 mM (1000 µg/mL).
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive control reported.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported		Explored but not found (only 2 concentration levels tested for most endpoints)
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 and most of the endpoints were tested at only 2 concentrations.		

1. Information on the study

Data point:	CA 5.5
Report author	Hao Y., Xu W. et al
Report year	2019
Report title	Roundup-Induced AMPK/mTOR-Mediated Autophagy in Human A549 Cells
Document No	Journal of agricultural and food chemistry (2019) Vol. 67(41), 11364-11372
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
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, POEA and a herbicidal formulation containing POEA as a co-formulant on the AMPK/mTOR signaling pathway was studied in human alveolar carcinoma A549 cells *in vitro*. Only the results of exposure to glyphosate at 100 µg/mL are reported and discussed in this summary. The endpoints selected to study the effect of glyphosate on autophagy are inhibition of viability, monodansylcadaverine (MDC) staining to mark autophagic vacuoles, visualization of double-membrane autophagosomes by TEM, autophagic flux, colocalization of mitochondria and lysosomes, opening of the mitochondrial permeability transition pore (mPTP), expression of proteins involved in the AMPK/mTOR signaling pathway, and ATP content. No effect could be demonstrated of glyphosate on any of these endpoints indicating that glyphosate, in contrast to POEA and Roundup, does not contribute to the activation of the AMPK/mTOR signaling pathway and has thus no role in autophagy.

This publication is relevant for the risk assessment of glyphosate but reliable with restrictions because only one glyphosate concentration was tested and no positive controls were used.

Reliability criteria for *in vitro* toxicology studies

Publication: Hao, Xu *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of ≥95%. Source: Weihai Hanfu Biochemical Pharmaceutical Co., Ltd. ,China.
Only glyphosate acid or one of its salts is the tested substance	N	Also GBH (Monsanto, St. Louis, USA) and POEA tested.
AMPA is the tested substance	N	
Study		

Test system clearly and completely described	Y	Human alveolar carcinoma A549 cell line.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Glyphosate only at one concentration tested: 100 µg/mL.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive control.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Only one concentration for glyphosate.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is relevant for the risk assessment of glyphosate but reliable with restrictions because only one glyphosate concentration was tested and no positive controls were used.		

1. Information on the study

Data point:	CA 5.4
Report author	Ilyushina N. A. *et al.*
Report year	2018
Report title	Comparative investigation of genotoxic activities of glyphosate technical products in the micronucleus test in vivo
Document No	Toksikologicheskii Vestnik, (2018) No. 4, pp. 24-28.
Guidelines followed in study	OECD Test Guideline 474
Deviations from current test guideline	Positive control animals were included but the data are not reported. No ratio of PCE to NCE was reported. No evidence of bone marrow exposure Data have been presented per group rather than per animal
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:

This paper describes the results of three different technical batches of glyphosate tested in a mouse micronucleus test. The study followings the recommendations of OECD 474, with some deficiencies, mostly regarding the reporting of data rather than test methodology. However, the reliability of the reported conclusions are unknown due to a lack of clarity and accuracy of the reported data. Individual animal data and group mean±SD frequency of micronucleated (MN) PCE have not been reported. Instead the results per group appear to be presented as the total number of MN PCE found per 40,000 PCE, together with the frequency of MN PCE values, expressed as relative to control, for the group with calculated 95% upper and lower limits (no explanation for how these data awere derived is provided). The text describes the vehicle control MN PCE frequencies as ranging between 0.06% and 0.12% (within HCD) but similar detail is not provided for the treated groups. Consequently, there is no indication of animal variability within the groups and it is unknown if any of the treated animals fall outside of HCD. Furthermore, the total number of MN PCE is described as being per 40,000 PCE, however, only 4000 PCE were scored per animal and with 5 animals per group this would result in total of 20,000 PCE per group.

The authors postulate that the positive results observed for technical batch I are likely to be due to the presence of 0.13% formaldehyde in the material, although they provide no data to support their hypothesis.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the source of the 3 glyphosate batches was not revealed although the concentrations of the most important impurities were given for each batch. Although reference was made to OECD test guideline 474 too little detail was given on the conduct of the MN assay to conclude to reliability without restrictions and the data are inadequately reported.

Reliability criteria for *in vivo* toxicology studies

Publication: Ilyushina *et al*., 2018	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		

Study in accordance to valid internationally accepted testing guidelines	Y	OECD test guideline 474 according to the authors.
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 from 3 sources with purity of 96.6%, 95.8%, and 95.7%. Sources were not reported but the concentration of impurities (nitroso-glyphosate and formaldehyde) was given.
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	CD-1 mouse.
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Oral by gavage.
Dose levels reported	Y	0, 500, 1,000, and 2,000 mg/kg bw twice, twenty-four hours apart.
Positive control	Y	Cyclophosphamide.
Number of animals used per dose level reported	Y	5/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	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	Y	Referred to but not presented.
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 source of the 3 glyphosate batches was not revealed although the concentrations of the most important impurities were given for each batch. Although reference was made to OECD test guideline 474 too little detail was given on the conduct of the MN assay to conclude to reliability without restrictions, and the data are inadequately reported.		

1. Information on the study

Data point:	CA 5.4
Report author	Ilyushina N. *et al.*
Report year	2019
Report title	Maximum tolerated doses and erythropoiesis effects in the mouse bone marrow by 79 pesticides' technical materials assessed with the micronucleus assay
Document No	Toxicology Reports (2019) Vol. 6, 105-110
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
Along with 51 other pesticides 4 batches of glyphosate with purities ranging from 95.1 to 98.3% were investigated for their effect on erythropoiesis in mice. To assess the toxicity of glyphosate on the bone marrow the *in vivo* micronucleus test in the mouse according to OECD test guideline 474 was conducted at the limit dose of 2,000 mg/kg bw. No effect of glyphosate on erythropoiesis was found.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the source of the glyphosate batches used was not reported and no suitable positive control was used in the micronucleus test to assess bone marrow toxicity. The test conducted was in compliance with OECD test guideline 474.

Reliability criteria for *in vivo* toxicology studies

Publication: Ilyushina *et al*., 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	Y	OECD TG 474, in vivo MN assay only used with the purpose to assess toxicity to erythropoiesis in the bone marrow. The positive control used (cyclophosphamide) is not suitable as a positive control for bone marrow toxicity.
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?	4 batches were tested with purity of 95.7, 98.3, 95.1, and 95.8%. Source was not

		mentioned.
Only glyphosate acid or one of its salts is the tested substance	N	51 other pesticides were tested as well.
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	Only limit dose of 2,000 mg/kg bw was considered.
Number of animals used per dose level reported	Y	At least 5 groups of minimum 5 mice per sex.
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?	Only effect on erythropoiesis was reported.
Statistical methods described	Y	
Historical control data of the laboratory reported	Y	
Dose-effect relationship reported	N	Not possible since only one dose was used.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the source of the glyphosate batches used was not reported and no suitable positive control was used in the micronucleus test to assess bone marrow toxicity. The test conducted was in compliance with OECD test guideline 474.		

1. Information on the study

Data point:	CA 5.6
Report author	Johansson H. K. L. *et al.*
Report year	2018
Report title	Exposure to a glyphosate-based herbicide formulation, but not glyphosate alone, has only minor effects on adult rat testis
Document No	Reproductive Toxicology (2018) Vol. 82, 25-31
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 effects of glyphosate on intra-testicular testosterone levels, expression of Leydig cell specific genes Cyp11a1, Cyp17a1, Insl3, Hsd3β1 and Star and expression of somatic marker gene Ar or germ cell marker gene Ddx4, expression of Leydig cell-specific steroidogenesis factors CYP11A1 and STAR, testicular histopathology and apoptosis were investigated in male rats treated orally at 0, 2.5 and 25 mg/kg bw/day for 2 weeks. No effects were found on either of the testicular parameters tested suggesting that glyphosate does not contribute to endocrine disrupting effects of the male reproductive system.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because only two dose levels were used to explore the dose-effect relationship for the endpoints assessed.

Reliability criteria for *in vivo* toxicology studies

Publication: Johansson *et al*., 2018.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity ≥ 96%. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance		Also formulations were tested: Glyfonova 450 Plus, FMC corporation.
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	Oral by gavage.
Dose levels reported	Y	Only 2 dose levels for glyphosate (2.5 and 25 mg/kg bw)
Number of animals used per dose level reported	Y	10 animals/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	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	Only 2 dose levels tested.
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 only two dose levels were used to explore the dose-effect relationship for the endpoints assessed.		

1. Information on the study

Data point:	CA 5.4
Report author	Kasuba V. *et al.*
Report year	2017
Report title	Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line
Document No	Environ Sci Pollut Res (2017) Vol. 24, 19267–19281
Guidelines followed in study	Not mentioned
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 examine the effects of acute exposure (4 and 24 hours) of human hepatoma HepG2 cells to glyphosate at concentrations equivalent to the systemic concentrations at the ADI, REL and OEL. The endpoints investigated in this study are cell proliferation, DNA damage, MN formation and oxidative stress. A non-statistically significant increase in cell proliferation was seen in the CCK-8 test with no dose-effect relationship. The comet assay showed a statistically significant decrease in tail intensity after 4 hours with no difference from control after 24 hours. In the CBMN cytome assay a non-statistically significant increase in BN MN frequency was seen after 4 hours without a dose-effect relationship. After 24 hours, a decrease instead of an increase in BN MN frequency was reported. The nuclear bud frequency was statistically significantly elevated after 4 hours of exposure but was statistically significantly lower than control after 24 hours of exposure. The indicator tests for oxidative stress did not show a substance related effect.

This publication is considered relevant but reliable with restrictions because the cytogenetic damage found *in vitro* at a systemic concentration corresponding with the ADI (0.5 µg/mL which should have been 0.17 µg/mL) was not confirmed in *in vivo* regulatory MN studies with doses up to 2000 mg/kg bw corresponding with a systemic concentration of about 50 µg/mL.

Reliability criteria for *in vitro* toxicology studies

Publication: Kasuba *et al.,* 2017	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines		
Study performed according to GLP		
Study completely described and conducted following scientifically acceptable standards		
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of ≤100% as Pestanal®. Source: Sigma-Aldrich Laborchemikalien GmbH, Germany.
Only glyphosate acid or one of its salts is the tested substance		As “Pestanal”
AMPA is the tested substance	N	

Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	Culture concentration not in correspondence with ADI of 0.5 mg/kg bw.
Metabolic activation system clearly and completely described	N	HepG2 cells used
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
Cytotoxicity tests reported	N	Very low concentrations used
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	?	No significant effects were observed for the level of ROS and GSH for 4h and 24h incubation, and for 24 h for lipid peroxidation. Elevated levels of cytogenetic damage were found in the CBMN assay and the comet assay results indicate possible cross-linking or DNA adduct formation. These data were obtained at in vitro test concentrations that correspond with an external dose of 0.5 mg/kg bw/day (ADI) whereas there are regulatory studies with no effects at doses up to 2,000 mg/kg bw/day
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant but reliable with restrictions because the cytogenetic damage found in vitro at a systemic concentration corresponding with the ADI (0.5 µg/mL which should have been 0.17 µg/mL) was not confirmed in *in vivo* regulatory MN studies with doses up to 2000 mg/kg bw corresponding with a systemic concentration of about 50 µg/mL.		

1. Information on the study

Data point:	CA 5.4
Report author	Koller V. J. *et al.*
Report year	2012
Report title	Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells
Document No	Arch Toxicol (2012) Vol. 86, 805–813
Guidelines followed in study	SCGE assays were performed according to the guidelines described by Tice et al. (2000) and the CBMN Cytome assay according to Fenech (2007). The corresponding Guidance Documents are OECD 489 and OECD 487
Deviations from current test guideline	Cell type (buccal cells)
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

The objective of the present study was to find out whether exposure of human-derived buccal epithelial TR146 cells to glyphosate and Roundup causes adverse effects. In cytotoxicity experiments, four different endpoints were used, which reflect different modes of action. To assess the effect of glyphosate and Roundup on DNA stability, single cell gel electrophoresis assays (SCGE) were conducted under standard alkaline conditions reflecting the formation of single- and double-strand breaks. Additionally cytokinesis-block MN cytome assays have been conducted in which different nuclear anomalies were measured. This study demonstrated that there is a big difference in cytotoxicity between glyphosate and Roundup. This is not surprising since the surfactants present in glyphosate formulations decrease the integrity of cell and mitochondrial membranes causing toxicity and ensuing DNA instability. Glyphosate was found to significantly increase tail intensity as of 20 mg/L but without any further increase with dose from 40 to 2000 mg/L. Roundup increased in a dose dependent manner the tail intensity from 20 mg glyphosate eq./L up to 200 mg glyphosate eq./L with increasing cytotoxicity and 0% cell integrity at 200 glyphosate eq. mg/L. This indicates that there is a relationship between the cytoxicity of Roundup and DNA instability. This study has demonstrated a greater sensitivity of buccal epithelial cells for glyphosate and its formulations than hematopoietic cells where no effects have been noted in *in vivo* MN tests with doses up to 2,000 mg/kg bw. Since there is no direct exposure of the buccal epithelium with the Roundup formulation (unless it is swallowed) during application and the inhalation of aerosol of the spray dilution during application is negligible (Jauhiainen A *et al*. (1991) Am. Ind. Hyg. Assoc. J. 52, 61–64) the likelihood of DNA damage in epithelial cells of the GI and the respiratory tract remains very low.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with most of the reliability criteria of an *in vitro* toxicology study.

Reliability criteria for *in vitro* toxicology studies

Publication: Koller *et al.*, 2012.	**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: Monsanto Europe S.A.
Only glyphosate acid or one of its salts is the tested substance	Y	Also formulation (Roundup Ultra Max) was 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	10-1000 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	Roundup, but not glyphosate causes pronounced cytotoxic effects in human-derived buccal epithelial cells. The alkaline comet test results show that glyphosate as well as Roundup induce comet formation that reflect strand breaks and apurinic sites. 20 µg/mL glyphosate in Roundup caused a 3-fold increase over the background, with glyphosate alone a weaker effect was seen.
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 of an *in vitro* toxicology study.		

1. Information on the study

Data point:	CA 5.9 Exposure study
Report author	Kongtip P. *et al.*
Report year	2017
Report title	Glyphosate and Paraquat in Maternal and Fetal Serums in Thai Women
Document No	Journal of Agromedicine 2017, vol. 22 no. 3, 282-289
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

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

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.i.	NA	Subjects of a pilot birth cohort being monitored for glyphosate and paraquat.
Exposure to formulations with glyphosate combined with other a.i.	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.		

1. Information on the study

Data point:	CA 5.3
Report author	Kumar S. *et al.*
Report year	2014
Report title	Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation
Document No	Toxicology 325 (2014) 42-51
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:

This study evaluated nose-only exposure to glyphosate and collected farm samples containing glyphosate and evaluated the immune response in the lungs. This is not a guideline study nor an endpoint used in risk assessment. This study in not usable for risk assessment in terms of hazard assessment. In terms of exposure, the study determined that average amount of glyphosate per filter from environmental samples after spray application to fields was 17.33 µg, which correspond to average airborne concentration of 22.59 ng/m^3. The method for the collection and analysis of the air samples was not validated and the assumptions and calculations used in the determination of the average airborne concentration were not provided, therefore the results cannot be verified. While the study itself is acceptable, it is unreliable in terms of usable endpoints for risk assessment.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the method for the collection and analysis of the air samples was not validated.

Reliability criteria for *in vivo* toxicology studies

Publication: Kumar *et al*., 2014.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity is reagent grade. Source: Sigma - Aldrich, St. Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Sampling of aerosols from field spraying also contain co-formulants of the GBH applied.

AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	Female mice of wild type and IL-13-/- BALB/c background.
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Intranasal application of air samples taken during glyphosate field application (24 hours) and glyphosate.
Dose levels reported	Y	Field air sample and 100 ng, 1 µg or 100 µg of glyphosate delivered intranasally.
Number of animals used per dose level reported	Y	
Method of analysis described for analysis test media	Y	Glyphosate measured in air sample with ELISA kit.
Positive control	Y	Ovalbumin.
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	For glyphosate.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the method for the collection and analysis of the air samples was not validated.		

1. Information on the study

Data point:	CA 5.4
Report author	Kwiatkowska M. *et al.*
Report year	2017
Report title	DNA damage and methylation induced by glyphosate in human peripheral blood mononuclear cells (in vitro study)
Document No	Food and Chemical Toxicology (2017) Vol. 105, 93-98
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:
It was the objective of this study to investigate the effect of high glyphosate concentrations on DNA integrity and DNA methylation in PBMCs *in vitro*. It was demonstrated that glyphosate increased statistically significantly DNA damage (single and double strand-breaks and alkali-labile sites formation) from 0.5 mM up to 10 mM. Repair of the DNA lesions was significant at all concentrations tested after 120 minutes of recovery. The percentage of the global DNA methylation level was statistically significantly decreased by glyphosate at 0.25 mM but not at 0.5 mM. On the contrary, p53 promoter region methylation was statistically significantly increased as compared to control cells at 0.25 and 0.5 mM.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the lowest concentration at which DNA damage was observed (0.5 mM) is higher than the blood concentrations in rats (0.3 mM) obtained after dosing at the limit dose of 2000 mg/kg bw where no MN effects were seen.

Reliability criteria for *in vitro* toxicology studies

Publication: Kwiatkowska *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: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	Salt not mentioned
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		
Test concentrations in physiologically acceptable range (< 1	Y/N	0.25 to 10 mM

mM)		
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	For DNA damage. The concentration at which DNA damage was observed is higher than the blood concentrations in rats obtained after dosing at the limit dose of 2000 mg/kg bw for the detection of MN in vivo. The results obtained are not corroborated by regulatory in vivo genotoxicity studies.
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 lowest concentration at which DNA damage was observed (0.5 mM) is higher than the blood concentrations in rats (0.3 mM) obtained after dosing at the limit dose of 2000 mg/kg bw where no MN effects were seen.		

1. Information on the study

Data point:	CA 5.8.1
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	Food and chemical toxicology (2020) Vol. 135, pp. 110888
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:	Yes/Reliable with restrictions

2. 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

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.		

1. Information on the study

Data point:	CA 5.4
Report author	Mañas F. *et al.*
Report year	2013
Report title	Oxidative stress and comet assay in tissues of mice administered glyphosate and AMPA in drinking water for 14 days
Document No	Journal of Basic & Applied Genetics (2013) Vol. 24(2), Article 7 - research
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 investigate the effect of glyphosate and AMPA on indicators of oxidative stress and DNA integrity in mice after oral exposure for 14 days via the drinking water. The results of this study indicate that no statistically significant differences have been found in liver, kidney, lung and heart for all oxidative stress parameters measured with the exception of a decrease in SOD activity in the heart and an increase in CAT activity in the kidney at a daily glyphosate dose of 400 mg/kg bw. There was an increase in CAT activity in the lung but this was not statistically significant and didn't show a dose-effect relationship. A statistically significant increase in DNA damage parameters was observed for glyphosate and AMPA with the exception of tail intensity in the liver for glyphosate at 40 mg/kg bw/day. No clear dose-effect relationship was evident for DNA damage parameters in blood after treatment with glyphosate. A dose-effect relationship was present for tail length and tail moment in the liver.

This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because the increased DNA damage seen (only 2 dose levels tested for glyphosate with too few animals) didn't show a dose-effect relationship in blood and occurred at dose levels (40 and 400 mg/kg bw/day) that are much lower than the 2000 mg/kg bw used in regulatory *in vivo* MN tests in the mouse with negative results.

Reliability criteria for *in vivo* toxicology studies

Publication: Manas et al., 2013	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	2 dose groups for glyphosate, one dose group for AMPA
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	Very old determination methods applied
Test substance		
Test material (Glyphosate) is sufficiently documented and	Y	Purity of 96%.

reported (i.e. purity, source, content, storage conditions)		Source: Sigma-Aldrich, Argentina.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	Y	Purity of 99%. Source: Sigma-Aldrich, Argentina.
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Exposure via drinking water
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	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported		Only 2 dose levels used. DNA damage (comet) at 40 and 400 mg/kg bw/day (no dose-effect relationship) not confirmed by regulatory genotoxicity (MN) studies up to 2000 mg/kg bw/day
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 the increased DNA damage seen (only 2 dose levels tested for glyphosate with too few animals) didn't show a dose-effect relationship in blood and occurred at dose levels (40 and 400 mg/kg bw/day) that are much lower than the 2000 mg/kg bw used in regulatory in vivo MN tests in the mouse with negative results.		

1. Information on the study

Data point:	CA 5.6
Report author	Manservisi F. *et al.*
Report year	2019
Report title	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
Document No	Environmental Health (2019) Vol.18, 15
Guidelines followed in study	Pilot study based on OECD test guideline 443: Extended one-generation reproductive toxicity study.
Deviations from current test guideline	Yes, only one dose level for each test item and insufficient number of animals per dose level used.
GLP/Officially recognised testing facilities	No, no GLP statement delivered.
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
In this pilot study the effect of glyphosate and its reference formulation Roundup Bioflow (MON 52276) at a dose of 1.75 mg glyphosate acid eq./kg bw/day on endocrine modulation was investigated in female rats during pregnancy and lactation and in male and female rats during lactation, the peripubertal period and adulthood. The endpoints explored were body weight, water and food consumption, gestational parameters, litter parameters, landmarks of sexual development, estrous cyclicity, gross and histopathology of reproductive and endocrine tissues, sperm parameters and serum and plasma hormone levels. MON 52276 exposure was associated with statistically significant increase of ano-genital distance in males and females, a delay of first estrous and increased serum testosterone in females and altered testosterone metabolism in both males and females. MON 52276 elicited more pronounced effects than glyphosate, which only increased statistically significantly anogenital distance during the peripubertal period. The statistically significant increase in TSH levels in glyphosate and MON 52276 treated rats was not associated with histopathological changes in the thyroid and thus of minor toxicological significance. The effect of glyphosate on ano-genital distance is not corroborated by a regulatory reproductive toxicology study where rats were exposed to much higher doses of glyphosate (> 1,000 mg/kg bw/day).

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the limited number of animals used per dose level and only one dose level was tested.

Reliability criteria for *in vivo* toxicology studies

Publication: Manservisi *et al.,* 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Pilot study based on OECD test guideline 443 but with deviations.
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	Y	Purity of > 99.5% as

reported (i.e. purity, source, content, storage conditions)		Pestanal™ . Source: Sigma-Aldrich, Milan, Italy.
Only glyphosate acid or one of its salts is the tested substance	N	Also representative formulated product tested. Roundup Bioflow (MON 52276, containing 360 g/L of glyphosate acid in the form of 480 g/L isopropylamine salt of glyphosate (41.5%), water (42.5%) and surfactant (16%)). Source: Consorzio Agrario dell'Emilia, Bologna, Italy.
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	Oral via the drinking water.
Dose levels reported	Y	1.75 mg glyphosate acid eq./bw/day.
Number of animals used per dose level reported	Y	Dams: 8/group. Offspring: 8 M + 8F/group (6-week cohort); 10 M + 10F/group (13-week cohort).
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	Not possible, only comparison between glyphosate and MON 52276.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered as relevant for the risk assessment of glyphosate but reliable with restrictions because of the limited number of animals used per dose level and only one dose level was tested.		

1. Information on the study

Data point:	CA 5.7
Report author	Martínez M. et al.
Report year	2018
Report title	Neurotransmitter changes in rat brain regions following glyphosate exposure
Document No	Environmental Research 161 (2018) 212 - 219
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:
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 was historical control to determine if changes in the levels of neurotransmitters were within historical controls. No positive control included either. Also no analytical verification of dose levels.

Reliability criteria for *in vivo* toxicology studies

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 historical control data for comparison, 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 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.		

1. Information on the study

Data point:	CA 5.7
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	Toxicology Letters (2019) Vol. 304, 39-49.
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
The effect of glyphosate, AMPA and glycine was investigated on he 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 unlikely present toxicity towards the blood-brain barrier, glyphosate and AMPA at 1 and 10 µM may increase the barrier permeability in BMECs monolayers, glyphosate may increase paracellular permeability in BMECs monolayers to fluorescein via partial disruption of tight junction complexes integrity, exposure to high levels of glycine or AMPA (100 µM) may impair glucose uptake and metabolism in BMEC monolayers via an alteration in GLUT1 expression and/or activity, exposure to high concentrations of glyphosate (100 µM) may impair neurovascular coupling, chronic exposure to low levels (0.1 µM) of glyphosate or AMPA failed to show any signs of neurotoxicity and that low concentrations (< 10 µM) of glyphosate and AMPA may not have detrimental effects on iPSC-derived neurons.

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

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.		

1. Information on the study

Data point:	CA 5.9 Exposure study
Report author	McGuire M. K. *et al.*
Report year	2016
Report title	Glyphosate and aminomethylphosphonic acid are not detectable in human milk
Document No	The American journal of clinical nutrition, (2016) Vol. 103, No. 5, pp. 1285-90.
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 breast milk and urine samples from lactating women were analyzed 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 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.i.		
Exposure to formulations with glyphosate combined with other a.i.		
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	

Publication: McGuire *et al*., 2016	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
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.		

1. Information on the study

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	Food and Chemical Toxicology (2017) Vol. 108, 30-42
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:

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, microarray 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

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.		

1. Information on the study

Data point:	CA 5.3
Report author	Mesnage R. *et al*
Report year	2018
Report title	Comparison of transcriptome responses to glyphosate, isoxaflutole, quizalofop-p-ethyl and mesotrione in the HepaRG cell line
Document No	Toxicology Reports (2018) Vol. 5, 819–826
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
Together with 3 other herbicide active ingredients (quizalofop-p-ethyl, isoxaflutole and mesotrione) the effect of glyphosate on the transcriptome and metabolome profile of differentiated HepaRG cells was investigated at 0.06, 6 and 600 µM. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles when compared with the other herbicides tested. A follow-up metabolomics analysis of HepaRG cells exposed to glyphosate at 0.06 µM revealed a significant decrease in the levels of long chain fatty acids (LCFAs) and polyunsaturated fatty acids (PUFAs). At the higher glyphosate concentrations of 6 and 600 µM, lower lipid levels were also observed but these did not reach statistical significance. It is not clear, however, how these findings from an *in vitro* tissue culture model can be translated to effects *in vivo*.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no positive control was used and no cytoxicity tests were performed to optimise the concentration range to be explored.

Reliability criteria for *in vitro* toxicology studies

Publication: Mesnage *et al*., 2018.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate (purity ≥ 96%). Source: Sigma-Aldrich, Gillingham, Dorset, UK.
Only glyphosate acid or one of its salts is the tested substance	N	Also three other pesticide active ingredients were tested (quizalofop-p-ethyl, isoxaflutole and

		mesotrione).
AMPA is the tested substance	N	
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.06, 6 and 600 µM.
Cytotoxicity tests reported	N	
Transcriptomics and metabolomics methods described	Y	
Positive and negative controls	N	No positive controls included.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no positive control was used and no cytoxicity tests were performed to optimise the concentration range to be explored.		

1. Information on the study

Data point:	5.8.2/016
Report author	Mesnage , R. *et al.*
Report year	2018
Report title	Ignoring Adjuvant Toxicity Falsifies the Safety Profile of Commercial Pesticides
Document No	doi.org/10.3389/fpubh.2017.00361
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
This review article is presenting information on unverified effects of a small subset of co-formulants in some Glyphosate-based formulations, with an emphasis on POEA. This co-formulant is a surfactant banned in the EU glyphosate-based products.
Literature review section reaffirms low toxicity of the active ingredient compared with formulated products. No new data presented.

1. Information on the study

Data point:	CA 5.3
Report author	Milic M. *et al.*
Report year	2018
Report title	Oxidative stress, cholinesterase activity, and DNA damage in the liver, whole blood, and plasma of Wistar rats following a 28-day exposure to glyphosate
Document No	Arh Hig Rada Toksikol (2018) Vol. 69, 154-168
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

Glyphosate was orally administered to male rats at 0.1, 0.5, 1.75 and 10 mg/kg bw for 28 days to investigate its effect on DNA damage, oxidative stress and cholinesterase activity. The endpoints of this study were DNA damage as measured in the alkaline comet assay, ROS in plasma and liver, lipid peroxidation in plasma and liver, GSH in plasma and liver, GSH-Px activity in whole blood and liver and total cholinesterase, acetyl cholinesterase and butyl cholinesterase activity in plasma. The results of the alkaline comet assays revealed a statistically significant increase in tail length and tail intensity in leucocytes and small and medium sized liver nuclei. With the exception of tail length of small sized liver nuclei no dose effect relationship was evident. Tail intensity of the leucocytes could not be assessed because of the very high variability of the results. From the results of the oxidative stress markers in plasma and liver and cholinesterase activity in plasma it can be concluded that there was no dose related effect.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the high variability of the results of the comet assay, although the conduct of this assay was in general compliant with OECD test guideline 489.

Reliability criteria for *in vivo* toxicology studies

Publication: Milic *et al*., 2018.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	Y	*In vivo* Comet assay was compliant with OECD TG 489.
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 ≤100 %). Source: PESTANAL®, a registered trademark of Sigma-Aldrich Laborchemikalien

		GmbH, Germany
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	Oral by gavage.
Dose levels reported	Y	0.1, 0.5, 1.75 and 10 mg/kg bw/day, positive and negative control included.
Number of animals used per dose level reported	Y	5 males per 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	Y	Limited to body weight, organ weight, DNA damage, oxidative stress and ChE activity.
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	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the high variability of the results of the comet assay, although the conduct of this assay was in general compliant with OECD test guideline 489.		

1. Information on the study

Data point:	CA 5.4
Report author	Nagy K. *et al.*
Report year	2019
Report title	Comparative cyto- and genotoxicity assessment of glyphosate and glyphosate-based herbicides in human peripheral white blood cells
Document No	Environmental Health (2018) Vol. 17, pp. 52/1-52/13
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:
This paper describes a well conducted comet assay using human lymphocytes as the test system to examine the genotoxicity and cytotoxicity of glyphosate and 3 commercial products containing glyphosate. No DNA damage was induced by analytical grade glyphosate. The 3 glyphosate products induced an increase in tail intensity in the comet assay only at highly cytotoxic concentrations, non-toxic concentrations induced no DNA damage.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized and no positive control was used.

Reliability criteria for *in vitro* toxicology studies

Publication: Nagy *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, content and storage conditions are not reported. Source: VWR International Kft, Debrecen, Hungary.
Only glyphosate acid or one of its salts is the tested substance	N	Also 3 GBH tested: Roundup Mega, Fozat 480, Glyfos.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Human mononuclear white blood cells.
Test conditions clearly and completely described	Y	Comet selection criteria were not stated. It is not stated if the

		slides were coded prior to scoring
Metabolic activation system clearly and completely described	Y	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	1, 10, 100, 250, 500, 750 and 1000 µM.
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	No HCD so it is unknown what degree of background variation is apparent in this test system. This is exacerbated by the use of a single set of control cultures
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 no positive control was used.		

1. Information on the study

Data point:	CA 5.5
Report author	Pahwa M. *et al.*
Report year	2019
Report title	Glyphosate use and associations with non-Hodgkin lymphoma major histological sub-types: findings from the North American Pooled Project
Document No	Scandinavian Journal of Work, Environment & Health. 2019;45(6):600–609
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Not applicable for epidemiologic studies
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

The main advantage of this pooled analysis compared with the previously published individual studies was to enable a more comprehensive analysis for glyphosate with regard to confounding factors and proxy respondents. In general, adjusting for use of 2,4-D, dicamba, and malathion reduced ORs for glyphosate. Analyses that excluded proxy respondents were generally similar to analyses that included them, though there were some instances, specifically for other NHL subtypes, where excluding proxies appreciably reduced the adjusted OR.

Left unaddressed in this pooled analysis is the intractable issue of case-recall bias in case control studies. Crump has shown in an analysis of all the case control studies that have reported ORs for glyphosate, including the studies in this pooled analysis, that results for all pesticides were markedly skewed toward positive associations (Crump K, Risk Analysis DOI: 10.1111/risa.13440). Crump noted particularly that the ORs for individual pesticides in the McDuffie et al. study (and 2 other studies not included in this pooled analysis) were nearly all greater than 1.0. He considered this evidence of case recall bias. Fundamentally, using self-reported exposure recollections from cases and controls violates the basic principle that data should be collected under equivalent circumstances for the groups to be compared (viz., cases and controls). That is impossible when pesticide recall is likely to be affected by their grievous illness for cases and not for controls. Accordingly, while this pooled analysis is an advance in understanding confounding by other pesticides and in assessing the impact of reporting by proxies (except in analysis where 50% of the subjects were excluded due to data limitations) in the 2 included studies, systematic error related to case recall bias remains an outstanding issue for interpreting the results for glyphosate.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because it concerns a pooled case control study which is subject to recall and selection bias. Notably, potential case-recall bias remains an unresolved issue in this pooled reanalysis.

Reliability Criteria: Epidemiology studies

Publication: Pahwa M. *et al.,* 2019	**Criteria met? Y/N/?**	**Comments**
Study Design		

Publication: Pahwa M. *et al.,* 2019	**Criteria met? Y/N/?**	**Comments**
Adequate study design given study objectives	Yes	Pooled reanalysis of 2 previously published studies.
Appropriate study population to address potential glyphosate-related health outcomes	Uncertain	Populations had very limited glyphosate exposure frequency.
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Uncertain	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	Yes	
Comparable participation by groups being compared	No	Much less participation by controls: example: McDuffie study – participation 67% for cases, 48% for controls.
Information provided by proxy respondents	Yes, substantial	31% for cases, 40% for controls in DeRoos study; 21% for cases, 15% for controls McDuffie study (per Chang & Delzell 2016)
Adequate statistical analysis	Yes	More comprehensive than the original publications regarding confounding & proxy responses. Data for 47% of subjects were missing for analyses by days of use per year and lifetime cumulative days of use.
Adequate consideration of personal confounding factors	Yes	Better than original studies.

Publication: Pahwa M. *et al.,* 2019	**Criteria met? Y/N/?**	**Comments**
Adequate consideration of potentially confounding exposures	Yes	Better than original studies
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Yes	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because illustrates bias toward positive findings for glyphosate in the original publications due to confounding and, in part, due to proxy responses. Recall bias unresolved. Missing data for 47% of subjects for analyses by days/year of use and lifetime cumulative days of use hinders interpretation of related results.		

1. Information on the study

Data point:	CA 5.6
Report author	Panzacchi S. *et al.*
Report year	2018
Report title	The Ramazzini Institute 13-week study on glyphosate-based herbicides at human equivalent dose in Sprague Dawley rats: study design and first in-life endpoints evaluation
Document No	Environmental Health (2018) Vol. 17, 52
Guidelines followed in study	Based on the National Toxicology Program's (NTP) Modified One-Generation Reproduction Study 2011.
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

In this study the general toxicity of glyphosate was compared against that of its reference formulation MON 52276 in pregnant rats and their progeny. Also the urinary excretion of glyphosate and AMPA was investigated. The test compounds were administered via the drinking water resulting in a daily dose of 1.75 mg glyphosate acid eq./ kg bw. The endpoints investigated were mortality, body weight, water and food consumption, and clinical signs in dams and offspring and litter data. There was no mortality and no statistically significant differences were observed among control, glyphosate, and MON 52276 groups in any of the endpoints investigated. Urinary concentrations of glyphosate and AMPA of rats treated with glyphosate at 1.75 mg/kg bw/day were comparable to those observed in rats treated with MON 52276 at 1.75 mg glyphosate acid eq. /kg bw/day. This indicates that the co-formulants in this glyphosate formulation have little influence on the oral bioavailability of glyphosate. In the treated rats, the majority of glyphosate was excreted in urine unchanged at levels of about 100-fold higher than that of AMPA and the mean urinary concentration of glyphosate increased with the duration of treatment.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because only one dose level for glyphosate and MON 52276 was considered, only 8 animals were used per dose and per sex and the method of analysis of glyphosate and AMPA in urine and its validation were not fully reported.

Reliability criteria for *in vivo* toxicology studies

Publication: Panzacchi *et al*., 2018	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Only a part of the reproductive toxicology study was reported, one dose level of glyphosate or MON 52276 was considered and 8 females per dose group were used.
Study performed according to GLP	N	
Study completely described and conducted following	Y	For the part that was

scientifically acceptable standards		reported.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate (purity of > 99,5%), Pestanal™ analytical standard purchased from Sigma-Aldrich (Milan, Italy).
Only glyphosate acid or one of its salts is the tested substance	N	The representative formulated product, Roundup Bioflow (MON 52276, containing 360 g/L of glyphosate acid in the form of 480 g/L isopropylamine salt of glyphosate (41.5%), water (42.5%) and surfactant (16%) was purchased from Consorzio Agrario dell'Emilia, Bologna, Italy.
AMPA is the tested substance	Y	Determined in urine of rats treated with glyphosate and MON 52276.
Study		
Test species clearly and completely described	Y	Male and female SD rats.
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Oral via drinking water.
Dose levels reported	Y	1.75 mg glyphosate acid eq./kg bw/day administered as glyphosate and as MON 52276.
Number of animals used per dose level reported	Y	8 virgin female SD rats per dose group.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Method of analysis described for analysis of urine	N	No details on the conduct of the method of analysis and no complete validation data set.
Complete reporting of effects observed	Y	Limited to body weight,
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	N	Not possible with one dose level of each test item.

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 only one dose level for glyphosate and MON 52276 was considered, only 8 animals were used per dose and per sex and the method of analysis of glyphosate and AMPA in urine and its validation were not fully reported.		