検索期間：2020年1月～6月

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 5.5/026
Report author	Crump, K. et al.
Report year	2020
Report title	Accounting for Multiple Comparisons in Statistical Analysis of the Extensive Bioassay Data on Glyphosate.
Document No	https://doi.org/10.1093/toxsci/kfaa039
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	-/Reliable without restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

Ten cancer bioassays of sufficient quality and which allowed the analysis of individual animal data was selected for the application of a multi-response permutation procedure that adjusts for the large number of tumors eligible for statistical testing and provides valid false-positive probabilities. The statistical tests applied in the analysis were functions of p-values obtained from conventional continuity-corrected poly-3 tests for trend applied to each type of tumor or combination of tumor types in each bioassay. Results from 3 multi-response permutation tests are reported and discussed: the "min-test", the "05-test" and the "01-test". In the min-test, the test statistic is the smallest p-value obtained from applying the poly-3 test to all tumor types in all bioassays investigated. Animals are randomly reassigned to dose groups in a Monte Carlo analysis, keeping the total numbers of animals in each dose group equal to the number in the original data. The tumors in each such reassignment are analyzed using the poly-3 test in exactly the same way as in the original data. Males and females are permuted separately. The false positive rate is the proportion of random reassignments that result in a smallest poly-3 p-value that is smaller than or equal to the smallest poly-3 p-value obtained from the original data. The test statistics for the 05-test and the 01-test are the number of poly-3 tests of tumors in the original data for which the p-value is less than or equal to the critical value of 0.05 or 0.01, respectively. In all applications of the poly-3 test, the test is applied only to data from one sex in a single study and the p-values from the poly-3 tests of all the studies are combined to create the "global" tests (min-test, 05-test and 01-test) to give the correct false positive rates. In addition to the randomization procedures for testing for positive dose-response trends in tumor incidence, the same procedures were repeated after reconfiguring the poly-3 test for negative trends. When the frequency of poly-3 p-values for positive trend computed from all tumors in all 10 bioassays in which at least two tumors occurred are considered there is an excess of large p-values (close to 1.0) compared to small p-values (close to 0.0). Since the version of the poly-3 trend test applied is a one-sided test for a positive trend, p-values close to 1.0 would translate into p-values near 0.0 for one-sided trend tests for anti-carcinogenicity. Results of tests for a dose-related decrease in survival in each study show that in none of the bioassays analyzed this test was statistically significant. Moreover, 4 of the datasets had p-values in excess of 0.95 which indicates a significant positive trend in survival with increasing dose. The most significant poly-3 trend in all 10 bioassays was found in the [redacted] *et al.* (1993) study for hemangiosarcoma in male mice with a p-value of 0.0013. The actual significance of this smallest p-value, which is the false positive rate for the min-test, was 0.26 based on the primary analysis, rather than the naive value of 0.0013. This means that 26 % of the randomizations of the 10 datasets gave a smallest p-value less than or equal to the smallest p-value obtained from the original data. Besides, the incidence in hemangiosarcomas (8 %) remained within the historical control range and no such tumors were identified in another mouse study at a dose level nearly 5 times of that used in the [redacted] *et al.* (1993) study. Overall, these findings suggest that, after accounting for the

number of statistical tests performed, there was no clear evidence of a positive dose-related trend in tumor occurrence. The 01-test for a negative trend was highly significant with a p-value of 0.002. These findings suggest stronger evidence for negative rather than positive dose-response trends in tumor occurrence. In all 10 bioassays investigated, the analysis made in this paper identified 24 tumors that exhibited a poly-3 positive trend with a p-value of less than or equal to 0.05. Nevertheless, after accounting for the multitude of statistical tests this analysis did not find that number statistically significant ($p = 0.08$). The statistical analysis of 10 glyphosate bioassays presented in this paper found no strong statistical evidence that glyphosate is carcinogenic. This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because state-of-the-art statistical methods were employed to a selected set of cancer bioassays to demonstrate false-positive probabilities.

Reliability criteria for *in vivo* toxicology studies

Publication: Crump *et al*., 2020.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N.A.	
Study performed according to GLP	N.A.	
Study completely described and conducted following scientifically acceptable standards	Y	Statistical re-analysis of 10 selected bioassays for which individual animal data are available.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Provided in the original bioassays.
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	Provided in the original bioassays.
Test conditions clearly and completely described	Y	Provided in the original bioassays.
Route and mode of administration described	Y	Oral *via* the diet.
Dose levels reported	Y	Provided in the original assays. The maximum doses reported are 5,873 mg/kg bw/day and 4,841 mg/kg bw/day in female and male mice, respectively.
Number of animals used per dose level reported	Y	50 – 64 animals per dose group.
Method of analysis described for analysis test media	N	Should be provided in original bioassays.
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	N.A.	Statistical re-analysis of all tumor sites.
Statistical methods described	Y	Application of a multi-response permutation procedure providing valid false-positive probabilities.
Historical control data of the laboratory reported	Y	For some tumors.
Dose-effect relationship 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 state-of-the-art statistical methods were employed to a selected set of cancer bioassays to demonstrate false-positive probabilities.		

1. Information on the study

Data point:	CA 5.6
Report author	Ganesan S. *et al*.
Report year	2020
Report title	Absence of glyphosate-induced effects on ovarian folliculogenesis and steroidogenesis
Document No	Reproductive Toxicology, (2020) 96, 156-164 DOI: 10.1016/j.reprotox.2020.06.011
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

In vivo study on post natal day 42, glyphosate administered to C57BL/6 J female mice at 0 or 2 mkd (10 mice/dose). Cyclicity, follicle number, circulating ovarian steroid hormone levels and ovarian intracellular signaling parameters were tested in adult female mice during 5 or 10 weeks. Glyphosate exposure for five or ten weeks did not affect the ovarian and endocrine endpoints examined.

The article is classified as reliable with restrictions for the following reasons: only 1 dose tested (no dose relationship can be evaluated), purity of glyphosate is not clear, method of analysis for analysis test media & no validation of the analytical method was described, no GLP status stated, no OECD guideline followed. Although no HCD were available in order to compare with the equivalent concurrent controls and test groups results, the results were negative and therefore did not require HCD to interpret or provide context to any findings.

Reliability Criteria: *In Vivo* Toxicology Studies		
Publication: Ganesan S. *et al*. 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	Not stated
Study completely described and conducted following scientifically acceptable standards	Y	Use of control group, 10 female mice/group, 1 dose + 1 negative control, statistical analysis performed, no randomisation of animals mentioned, no HCD
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate No Purity, no storage condition stated
Only glyphosate acid or one of its salts is the tested substance	N	Glyphosate
AMPA is the tested substance	N	
Study		

Reliability Criteria: *In Vivo* Toxicology Studies		
Publication: Ganesan S. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Test species clearly and completely described	Y	C57BL/6 J mice, age, origin stated but initial BW not included
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Per os
Dose levels reported	Y	0 and 2 mg/kg/d; 5 days/week for 5 and 10 weeks
Number of animals used per dose level reported	Y	10 mice/group 5 weeks (n = 10 control; n = 10 GLY) and 10 weeks (n = 10 control; n = 10 GLY)
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	N	Only 1 dose tested
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	only 1 dose tested (no dose relationship can be evaluated), purity of glyphosate is not clear, method of analysis for analysis test media & no validation of the analytical method was described, no GLP status stated, no OECD guideline followed. Although no HCD were available in order to compare with the equivalent concurrent controls and test groups results, the results were negative and therefore did not require HCD to interpret or provide context to any findings.
Not reliable	N	

1. Information on the study

Data point:	CA 5.8.3
Report author	Gastiazoro M.P. *et al.*
Report year	2020
Report title	Glyphosate induces epithelial mesenchymal transition-related changes in human endometrial Ishikawa cells via estrogen receptor pathway
Document No	Molecular and cellular endocrinology, (2020), Vol. 510, Art. No. 110841
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes / Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

Ishikawa endometrial cancer cells were treated with glyphosate at 0.2 µM and 2 µM. Glyphosate caused cell migration, invasion ability and down regulated E-cadherin mRNA expression. 17ß-estradiol, which was included as a positive control caused similar epithelial mesenchymal transition related changes, while treatment with fulvestrant (estrogen receptor antagonist) reversed the effects caused by glyphosate. The findings suggest that glyphosate has the ability to trigger the estrogen receptor-dependant pathway.

The relevance to human health risk assessment of this unvalidated *in vitro* research model in an immortal adenocarcinoma cell line containing estrogen and progesterone receptors is not clear. The results contradict a number of higher tier studies conducted across a variety of test systems,

The article is classified as reliable with restrictions for the following reason : glyphosate was tested at two different concentrations only, no test guideline was used and no historical control data were provided in order to compare with the equivalent concurrent controls and test groups results. Further, the study was not performed according to GLP.

Reliability criteria for *in vitro* toxicology studies		
Publication: Gastiazoro M.P. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	Not stated
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate (purity ≤ 100%)
Only glyphosate acid or one of its salts is the tested substance	N	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Ishikawa cells

Reliability criteria for *in vitro* toxicology studies		
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	Not necessary
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.2 µM and 2 µM
Cytotoxicity tests reported	Y	Viability was assessed with tryptan blue dye exclusion assay
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	Y	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	Glyphosate was tested at two different concentrations only, no test guideline was used and no historical control data were provided in order to compare with the equivalent concurrent controls and test groups results. Further, the study was not performed according to GLP.
Not reliable	N	

1. Information on the study

Data point:	CA 5.2.6
Report author	Lindberg T. *et al.*
Report year	2020
Report title	An integrated transcriptomic- and proteomic-based approach to evaluate the human skin sensitization potential of glyphosate and its commercial agrochemical formulations
Document No	Journal of proteomics, (2020) Vol. 217, pp. 103647
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes / Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
Investigation of molecular mechanisms in the skin sensitization process specifically focusing on DC activation using an integrated transcriptomic- and proteomic approach.
First, Mutz-3-derived cells were exposed to PPD, DMSO, unexposed sample and glyphosate. No cytotoxicity was observed for glyphosate and glyphosate was classified as non-sensitising.
Second, PPD, DMSO, unexposed sample and glyphosate were assembled to protein groups. A clear separation between sensitizers (PPD) and non-sensitizers (unexposed, DMSO, glyphosate) was observed. Data on glyphosate are consistent with other available validated assay results.

The article is classified as reliable with restrictions for the following reason: This is a non-validated test system. The purity and origin of glyphosate is unclear. only 1 dose tested (no dose relationship can be evaluated), no HCD were available in order to compare with the equivalent concurrent controls and test groups results.

Reliability Criteria: *In Vitro* Toxicology Studies		
Publication: Lindberg T. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Not applicable because there is no OECD guideline for this type of test
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	The only information is that glyphosate's input concentration was 500 µM. The purity, origin etc. is unclear.
Only glyphosate acid or one of its salts is the tested substance	N	Glyphosate alone and EU non-representative formulations were tested (summary above is provided only for glyphosate)
AMPA is the tested substance	N	

Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	n.a.	Not applicable as no metabolic activation system used
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Not applicable as only 1 concentration was used
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	This is a non-validated test system. The purity and origin of glyphosate is unclear. Only 1 dose tested (no dose relationship can be evaluated). No HCD were available in order to compare with the equivalent concurrent controls and test groups results.
Not reliable	N	

1. Information on the study

Data point:	CA 5.5/027
Report author	Portier, C.J.
Report year	2020
Report title	A comprehensive analysis of the animal carcinogenicity data for glyphosate from chronic exposure rodent carcinogenicity studies
Document No	Environ Health (2020) Vol. 19, 18. https://doi.org/10.1186/s12940-020-00574-1
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	-/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

Thirteen glyphosate cancer bioassays considered acceptable for this re-analysis were selected from the published literature, the EPA review, the review from the German Institute for Risk Analysis, the JMPR review, and full laboratory reports. For twelve of them full study reports were available. Individual tumour counts for the individual studies were re-analyzed using the exact Cochran-Armitage one-sided linear trend test. Re-analyses were conducted on all primary tumours where there were at least 3 tumours in all of the animals in a sex/species/strain combination. In addition, any tumour where a significant positive trend ($p \leq 0.05$) was found in at least one study was also evaluated in all the other studies of the same sex/species/strain combination, regardless of the number of animals with the tumour. Pairwise comparisons between individual exposed groups and the control group were conducted using Fisher's exact test. To evaluate the consistency of a tumour finding across multiple studies using the same sex-species-strain combinations, logistic regression with individual background responses and dose trends are fit to the pooled data using maximum likelihood estimation. The same methods of analysis were used to evaluate the incidence of non-neoplastic lesions in tissues where tumours were observed. In cases of rare tumours where the increase in incidence didn't reach statistical significance the test proposed by Tarone (1982) was applied using an appropriate historical control group. To summarize the results of the strength-of-evidence analysis, each tumour is placed in any of the following categories: Clear evidence (CE), some evidence (SE), equivocal evidence (EE), and no evidence (NE). The factors used to place tumours into these categories include the analyses of the individual studies, the consistency of the data across studies (pooled analyses), the analyses using historical control data, the analyses of non-neoplastic lesions, and mechanistic evidence with the associated scientific literature. The author's weight-of-evidence analysis indicates that there is clear evidence (CE) that oral exposure to glyphosate *via* the diet produces adrenal cortical carcinoma in the female SD rat, hemangioma in the female mouse (CD-1 and Swiss albino), hemangiosarcoma in the male CD-1 mouse, kidney tumours in the male CD-1 mouse and SD rat, liver adenoma in the male rat (SD and Wistar), malignant lymphoma in the male and female CD-1 mouse, skin basal cell tumour in the male SD rat and skin keratoacanthoma in male rats (SD and Wistar). Some evidence (SE) for a causal relationship was put forth for kidney tumours in the male Swiss albino mouse, mammary tumours in the female Wistar rat, malignant lymphoma in the male and female Swiss albino mouse, pituitary adenoma in the male and the female Wistar rat, and testicular interstitial cell tumours in the male SD rat.

After thorough analysis and considering all factors that are important in the interpretation of cancer studies none of the tumours identified by the author as indicating clear evidence (CE) or some evidence (SE) of carcinogenicity were found relevant for reconsideration under the necessary due diligence of the European AIR5 review of glyphosate. Most of the tumours selected by the author were previously dismissed by the EU experts as not relevant even before the last review of glyphosate

in 2017, and the applicant believes that there is no solid toxicological evidence for glyphosate exposure related carcinogenicity in the mouse and the rat that warrants any science-based concerns for human health. The discussion of each of the suspect tumours is given below.

Clear evidence (CE) for carcinogenicity:

Adrenal cortical carcinoma in the female SD rat ([redacted] study):

The tumour incidences were 0/60, 0/60, 0/60, 3/60 at 0, 113, 457, and 1183 mg/kg bw/day, respectively. This tumour has not been considered treatment related by the authors of the study. There is no dose-related increase in adrenal cortical adenoma (1/60, 3/60, 2/60, 1/60), no dose-related increase in pre-neoplastic lesions and this tumour was not found in the males of the same study or in other rat studies. Therefore, this tumour has been considered not relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hemangioma in female CD-1 mice ([redacted] study):

In the [redacted] study hemangiomas were observed in different tissues:

- In liver with an incidence of 0/50, 0/50, 1/50, 1/50;
- In the ovary with an incidence of 0/50, 0/50, 0/50, 1/50;
- In the uterus with an incidence of 0/50, 0/50, 1/50, 2/50;
- In the spleen with an incidence of 0/50, 0/50, 1/50, 0/50;
- In the abdominal cavity with an incidence of 0/8, 0/9, 0/9, 1/9;

At 0, 153.2, 786.8, and 4116 mg/kg bw/day, respectively. Taken together as systemic tumours a significant positive trend is obtained. However, hemangiomas have also been observed in males (liver and testes) but without any dose-response relationship and the highest incidence found was in the control group (1/50). These tumours have not been confirmed in the other carcinogenicity studies in the CD-1 mouse. Moreover, the dose level (4116 mg/kg bw/day) at which the incidence was statistically significantly increased when compared against the control, is more than 4-fold the limit dose for the testing of carcinogens in rodent species. If that dose is ignored there is no significative positive trend. Therefore, this tumour has been considered not relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hemangioma in female Swiss albino mice ([redacted] study):

In the re-analysis of the tumour data of the [redacted] study by [redacted] (report submitted in 2017) no statistically significant trend was found for systemic neoplasms using the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Hemangiosarcoma in the male CD-1 mouse ([redacted] study):

The tumour incidences were 0/50, 0/50, 0/50, 4/50 (3 in liver and 1 in prostate) at 0, 98, 297, and 988 mg/kg bw/day, respectively. The incidence at the highest dose (8 %) was still within the historical control range of the test laboratory (0-8 %, 300 male mice in 6 studies up to 1993). This tumour was not confirmed in other mouse studies of which one ([redacted] study) with a dose level nearly 5-fold that of the [redacted] study (4841 mg/kg bw/day). Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Kidney tumours in the male CD-1 mouse ([redacted] study, as reported by JMPR, 2016):

Renal cell adenoma (3/50) and renal cell carcinoma (1/50) were observed in males at 7470 mg/kg bw/day, but, according to the authors, there was no statistically significant difference with the control group. It is of note that the high dose considered in this study for males is extraordinarily high, more than 7-fold the limit dose for the testing of carcinogens in rodent species. If this dose is ignored there is no significant positive trend. These tumours were re-examined by the original study pathologist in 2012 because the Pesticide Expert Panel of the Food Safety Commission of Japan requested more information on historical control data and association with the non-neoplastic renal findings. After re-examination, the incidences for renal cell adenoma were 1/50, 1/50, and 1/50 at 167.6, 685, and

7470 mg/kg bw/day, showing no dose-response relationship. The incidence for renal cell carcinoma was confirmed to be 1/50 at 7470 mg/kg bw/day. The historical control data for the [redacted] study were not available, but the historical control values described in the re-examination document for renal cell carcinoma were 1/725 (0.13 %) in males and 0/725 (0 %) in females and for renal cell adenoma were 3/564 (0.53 %) in males and 0/564 (0 %) in females. The re-examination report also provides reference data of 0-1.8 % in males and 0 % for all doses in females for renal cell carcinoma, and 0-1.8 % in males and females for renal cell adenoma. The results of the re-examination revealed also that the tubular epithelial cell hypertrophy was localized with an incidence in each treatment group that did not significantly differ from that in the control group. There was no association between the tubular epithelial cell hypertrophy and the development of renal tumours. The renal cell tumours observed in this study are thus not relevant for the human risk assessment of glyphosate because (1) the incidence of renal tumours in males at 7470 mg/kg bw/day did not significantly differ from that in the control group upon re-evaluation; (2) none of the females had neoplastic or non-neoplastic lesions; and (3) the high dose considered in this study for males is more than 7-fold the limit dose for the testing of carcinogens in rodent species. Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Kidney tumours in the male SD rat ([redacted] study):
The incidences of kidney adenoma were 0/76, 0/75, 0/80, 4/78 at 0, 104, 354, and 1127 mg/kg bw/day. An increasing trend in the incidence of adenomas in the kidney was observed in males of the high dose group (animal 193: killed in extremis at week 92, animal 167: found dead at week 94, animal 159: found dead at week 101, and animal 169: killed by design after 104 weeks of treatment) and this incidence was greater than the historical control range referred to in the study report (0-2.9 %). However, according to the authors of this study, the increase observed was not statistically significant. No kidney tumours were found in the females and nearly all male rats at all dose levels suffered from chronic nephropathy (62/76, 63/75, 56/80, 67/78). This tumour in this study was not considered relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hepatocellular adenomas in the male SD rat ([redacted]):
The tumour incidences for adenomas were 3/60, 2/60, 3/60, 8/60 and of carcinomas were 3/60, 2/60, 1/60, 2/60 at 0, 89, 362, and 940 mg/kg bw/day, respectively. The incidence of adenomas at the high dose (13.3 %) is still within the historical control range of the test laboratory (1.4-18.3 %). Foci of cellular alteration were observed at all dose levels without any dose-response relationship and there were no signs of hepatocellular hypertrophy, a prerequisite for hepatocellular carcinogenesis. Beside the [redacted] study no increase in hepatocellular adenomas was noted in the other rat studies. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Hepatocellular adenomas in the male Wistar rat ([redacted] study):
The tumour incidences were 0/64, 2/64, 0/64, 5/64 at 0, 121, 361, 1214 mg/kg bw/day, respectively. The positive trend is significant and the incidence at the high dose is significantly different from the control. However, the incidence at the high dose (7.8 %) is still within the historical control range of the test laboratory (0-11.5 %, 26 studies in 1984-2003). There were no histopathological signs of liver enzyme induction or pre-neoplastic lesions. The high dose animals in this study survived longer when compared to the other groups. This may also influence the spontaneous tumour rate. Beside the [redacted] study no increase in hepatocellular adenomas was noted in the other rat studies. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the male CD-1 mouse ([redacted] study):
The tumour incidences were 2/50, 2/50, 0/50, 6/50 at 0, 165, 838, 4348 mg/kg bw/day, respectively. The positive trend is significant but the incidence at the high dose is not significantly different from the control. Moreover, the incidence at the high dose (12 %) is still within the historical control range of the test laboratory (3.6-19.2 %, 458 male mice in 12 studies in 1993-1998). The trend has been found significantly positive because of the elevated incidence at a dose level that is over 4-fold the limit dose for carcinogenicity studies in rodents. If this dose is ignored the trend is not positive. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the female CD-1 mouse ([redacted] study):
The tumour incidences were 3/50, 1/50, 4/50, 6/50 at 0, 93.2, 909, and 8690 mg/kg bw/day, respectively. The increased incidence of lymphoma at the high dose was statistically significant in the trend test but not in a pairwise comparison. The trend has been found significantly positive because of the elevated incidence at an extraordinarily high dose level, more than 8-fold the limit dose for carcinogenicity studies in rodents. If this dose is ignored the trend is no longer significant. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Skin basal cell tumour in the male SD rat ([redacted] study):
The tumour incidences were 0/78, 0/75, 0/80, 3/78 for adenoma and 0/78, 0/75, 0/80, 1/78 for carcinoma at 0, 104, 354, and 1127 mg/kg bw/day. No increased incidence of this tumour was observed in the females or other rat studies and may be associated with other skin lesions (follicular hyperkeratosis and/or folliculitis/follicular abscess) observed in this study. Although there is a significant positive trend for the adenomas, the increase in incidence at the high dose level was not considered relevant for the risk assessment of glyphosate by the authors of this study. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat ([redacted] study):
The tumour incidences were 1/60, 3/60, 4/60, 5/60 at 0, 89, 362, and 940 mg/kg bw/day. Although there is a significant positive trend the incidence at the high dose was not statistically significantly different from the control and considered not related to treatment. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats. Therefore, this tumour was not considered relevant for the risk assessment of glyphosate by the authors of this study. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat ([redacted] study):
The combined incidences of intracutaneous cornifying epithelioma (keratoacanthoma) were 1/50, 2/25, 0/19, 0/21, 5/50 at 0, 11, 112, 320, and 1147 mg/kg bw/day. Although the trend was significant, the incidence at the high dose was not statistically significantly different from the control and considered not related to treatment by the authors of this study. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat ([redacted] study):
The incidences of the tumour were 4/76, 3/75, 0/80, 7/78 at 0, 104, 354, and 1127 mg/kg bw/day. The increased incidence of this skin tumour at the high dose may be associated with other skin lesions (follicular hyperkeratosis and/or folliculitis/follicular abscess) observed in this study. Although there is a significant positive trend for this tumour, the increase in incidence at the high dose level was not statistically significantly different from the control. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats and considered by the authors of this study not relevant for the risk assessment of glyphosate. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male Wistar rat ([redacted] study):
There were no treatment-related conditions seen in the skin or in subcutaneous tissues, but several spontaneous lesions were observed. Epidermal ulceration and scab formation, inflammatory lesions, abscess formation, focal acanthosis, focal mineralisation, focal dermal thickening, and focal necrosis were seen, occasionally or rarely and without significance. This tumour was not discussed further in the previous EU review of glyphosate.

Some evidence for carcinogenicity (SE)
Kidney tumours in the male Swiss albino mouse ([redacted] study):
In the re-analysis of the tumour data of the [redacted] study by [redacted] (submitted in 2017) no statistically significant trend was found for systemic neoplasms in the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise

comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Mammary tumour in the female Wistar rat ([redacted] study):
At interim and terminal sacrifice combined mammary neoplasia was seen in 6 female mice. There were no mammary neoplasms in the control group but carcinomas were seen with incidences of 2/51, 3/51, and 1/51 at 153.2, 786.8, and 4116 mg/kg bw/day, respectively. All neoplasms were adenocarcinomas with the exception of one adenosquamous carcinoma seen in a low dose group animal. No increase in the incidence of these tumours was reported in the females of other rat studies. The authors concluded that there was no effect of treatment upon the incidence of mammary neoplasia in this study. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the male Swiss albino mouse ([redacted] study):
In the re-analysis of the tumour data of the [redacted] study by [redacted] (2017) no statistically significant trend was found for systemic neoplasms in the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour was not considered as relevant for the assessment of glyphosate.

Pituitary adenomas in the male and the female Wistar rat ([redacted] study):
Pituitary adenomas were only seen in female mice with incidences of 0/51, 1/51, 0/51, 2/52 at 0, 104.5, 348.6, and 1381.9 mg/kg bw/day. The group distribution was unrelated to treatment. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Testicular interstitial cell tumour in the male SD rat ([redacted] study):
The incidences of this tumour were 0/50, 3/50, 1/50, 6/50 at 0, 3.05, 10.30, and 31.49 mg/kg bw/day, respectively. The positive trend is statistically significant and the incidence at the high dose level (12 %) is statistically significantly different from the control and greater that the historical control rate of the test laboratory (3.4-6.6 %). However, there was no dose-response relationship for interstitial cell hyperplasia (1/50, 1/50, 1/50, 0/50). Since the dose range considered in this study (0-31.5 mg/kg bw/day) is approximately at least 30-fold lower than that of all the other studies in rats where no increase of such tumours was found this finding should be considered as spontaneous in nature. Therefore, this tumour was not considered relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because some of the statistical methods employed were not described in sufficient detail. Besides, the results of this study are not in agreement with the findings of Crump *et al*. 2020 in relation to the estimation of false positives and the overall evaluation of the significance of the tumours by the EU regulatory authorities. All the tumours that were identified by the author as providing clear evidence for the carcinogenicity of glyphosate have been previously dismissed in the EU regulatory process.

Reliability criteria for *in vivo* toxicology studies

Publication: Portier, 2020.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N.A.	
Study performed according to GLP	N. A.	Most of the study reports analysed were GLP compliant.

Study completely described and conducted following scientifically acceptable standards	Y	Re-analysis of the tumour data of 13 selected glyphosate cancer bioassays.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of glyphosate used in every cancer bioassay mentioned.
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	N.A.	Described in the test reports of 12 of the selected studies. The data from one study were derived from a JMPR review.
Route and mode of administration described	Y	Oral *via* the diet.
Dose levels reported	Y	Dose range from 71.4 to 8690 mg/kg bw in the mouse and from 3.05 to 4348 mg/kg bw in the rat.
Number of animals used per dose level reported	Y	About 50 per dose group.
Method of analysis described for analysis test media	N.A.	Described in the original test reports.
Validation of the analytical method	N.A.	
Analytical verifications of test media	N.A.	
Complete reporting of effects observed	Y	
Statistical methods described	Y	All statistical methods used in the re-analysis of the tumour data were reported, however sometimes not in sufficient detail.
Historical control data of the laboratory reported	N.A.	
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 some of the statistical methods employed were not described in sufficient detail. Besides, the results of this study are not in agreement with the findings of Crump *et al*. 2020 in relation to the estimation of false positives and the overall evaluation of the significance of the tumours by the EU regulatory authorities. All the tumours that were identified by the author as providing clear evidence for the carcinogenicity of glyphosate have been dismissed in the EU regulatory process.		

1. Information on the study

Data point:	CA 5.8.3
Report author	Xia Y. *et al*.
Report year	2020
Report title	The endoplasmic reticulum stress and related signal pathway mediated the glyphosate-induced testosterone synthesis inhibition in TM3 cells
Document No	Environmental Pollution, (2020) 260, 113949 DOI: 10.1016/j.envpol.2020.113949
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

In vitro study on the effects of glyphosate on testosterone secretion and the role of endoplasmic reticulum stress in the process were investigated in TM3 cells. Results showed that exposure to glyphosate at concentrations below 200 mg/L had no effect on cell viability, while glyphosate at concentrations above 0.5 mg/L could inhibit the testosterone secretion in TM3 cells. Treatment of TM3 cells with glyphosate at 5 mg/L not only reduced the protein levels of testosterone synthase StAR and CYP17A1 but also inhibited testosterone secretion.

The article is classified as reliable with restrictions for the following reason: not enough information on the tested material (purity) was provided, no positive controls were used, and no statistical methods were described. Furthermore, no OECD guidelines were followed, no GLP status was stated, and no historical control data (HCD) were provided to compare the relevance of data. In addition, key literature in disagreement with the authors' findings appear to have been disregarded, suggesting bias within the research and the following publications: Hecker (2011), OECD validation of the H295R steroidogenesis assay with glyphosate; Levine (2007), demonstrating a lack of effect of glyphosate on the StAR protein; US EPA (2015), glyphosate EDSP weight of evidence evaluation; and EFSA (2017), peer review of glyphosate potential endocrine-disrupting properties.

Hecker M et al (2011), The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter-laboratory validation study, Environmental Science and Pollution Research 18(3):503-15

Levine S. L. Et al. (2007), Disrupting mitochondrial function with surfactants inhibits MA-10 Leydig cell steroidogenesis, Cell Biol Toxicol (2007) 23:385-400

US EPA (2015), EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals - EDSP: WEIGHT OF EVIDENCE ANALYSIS OF POTENTIAL INTERACTION WITH THE ESTROGEN, ANDROGEN OR THYROID PATHWAYS - CHEMICAL: GLYPHOSATE

EFSA (2017): Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate, Question number: EFSA-Q-2016-00663

Reliability Criteria: *In Vitro* Toxicology Studies		
Publication: Xia Y. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	Not stated
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate Purity not stated
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	N	TM3 cells (no purity stated)
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	Not necessary
Test concentrations in physiologically acceptable range (<1 mM)	N	0.01 to 200 mg/L
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive controls
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	Not enough information on the tested material (purity), no positive controls were used, no statistical methods were described. Furthermore, no OECD guideline followed, no GLP status was stated. In addtion, no HCD were available in order to compare the relevance of the data. In addition, key literature in disagreement with the authors' findings appear to have been disregarded, suggesting bias within the research and publication: Hecker (2011) OECD validation of the H295R steroidogenesis assay with glyphosate; Levine (2007) demonstrating a lack of effect of glyphosate on the StAR protein; US EPA (2015) glyphosate EDSP weight of evidence evaluation; EFSA (2017) peer review of glyphosate potential endocrine-disrupting properties.

Reliability Criteria: *In Vitro* Toxicology Studies		
Publication: Xia Y. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Not reliable	N	

1. Information on the study

Data point:	CA 5.8.2
Report author	Yahfoufi Z. A. *et al.*
Report year	2020
Report title	Glyphosate Induces Metaphase II Oocyte Deterioration and Embryo Damageby Zinc Depletion and Overproduction of Reactive Oxygen Species
Document No	Toxicology, (2020) Vol. 439, Art. No. 152466
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 quality of metaphase II noncumulus oocytes and embryos from mice were investigated following glyphosate exposure at different concentrations (max 300 μM). The concentrations were in the range of those found in human blood following accidental acute exposure or suicidal attempts.

Results indicate that glyphosate provokes disruption of the microtubule organizing center and chromosomal disorganization at the mid-position of the spindle due to spindle disappearance, and defective chromosomal alignment as well as depletion of intracellular zinc bioavailability and enhancement of reactive oxygen species (ROS) accumulation in the mouse oocytes. In the embryos (not specified the source and the embryonal stage) zinc depletion and accumulation of ROS was also observed in a dose-related manner.

The article is classified as *reliable with restrictions* for the following reason: Not performed according to GLP or an OECD test guideline. No purity of the test substance stated. No information of the source and the embryonal stage of the embryos were provided. There were no concurrent positive control or substances known to deteriorates oocyte quality through disassembly of microtubule organizing centers (like peroxynitrite) or ROS accumulation (like hydrogen peroxide, and hypochlorous acid) or dimercapto-1-propanesulfonic acid (DMPS) for zinc depletion.

Reliability criteria for *in vitro* toxicology studies		
Publication: Yahfoufi Z. A. et al. 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	No GLP or OECD test guideline followed
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	No purity, content, storage conditions
Only glyphosate acid or one of its salts is the tested substance	Unknown	
AMPA is the tested substance	N	
Study		

Reliability criteria for *in vitro* toxicology studies		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	No metabolic activation system present
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Max. concentration 300 µM
Cytotoxicity tests reported	N	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	Not performed according to GLP or an OECD test guideline. No purity of the test substance stated. No appropriate information on the tested embryos was provided. Tested dose correspond to level of glyphosate that may be found in human blood following human accidental acute exposure intoxication. No reference compound were tested in parallel to confirm the appropriateness of the testing procedures and the relevance of the results.
Not reliable	N	

検索期間：2020年1月～6月

区分ａに分類された文献とその理由

農作物及び畜産物への残留

1. Information on the study

Data point:	CA 5.5/026
Report author	Crump, K. et al.
Report year	2020
Report title	Accounting for Multiple Comparisons in Statistical Analysis of the Extensive Bioassay Data on Glyphosate.
Document No	https://doi.org/10.1093/toxsci/kfaa039
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	-/Reliable without restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

Ten cancer bioassays of sufficient quality and which allowed the analysis of individual animal data was selected for the application of a multi-response permutation procedure that adjusts for the large number of tumors eligible for statistical testing and provides valid false-positive probabilities. The statistical tests applied in the analysis were functions of p-values obtained from conventional continuity-corrected poly-3 tests for trend applied to each type of tumor or combination of tumor types in each bioassay. Results from 3 multi-response permutation tests are reported and discussed: the "min-test", the "05-test" and the "01-test". In the min-test, the test statistic is the smallest p-value obtained from applying the poly-3 test to all tumor types in all bioassays investigated. Animals are randomly reassigned to dose groups in a Monte Carlo analysis, keeping the total numbers of animals in each dose group equal to the number in the original data. The tumors in each such reassignment are analyzed using the poly-3 test in exactly the same way as in the original data. Males and females are permuted separately. The false positive rate is the proportion of random reassignments that result in a smallest poly-3 p-value that is smaller than or equal to the smallest poly-3 p-value obtained from the original data. The test statistics for the 05-test and the 01-test are the number of poly-3 tests of tumors in the original data for which the p-value is less than or equal to the critical value of 0.05 or 0.01, respectively. In all applications of the poly-3 test, the test is applied only to data from one sex in a single study and the p-values from the poly-3 tests of all the studies are combined to create the "global" tests (min-test, 05-test and 01-test) to give the correct false positive rates. In addition to the randomization procedures for testing for positive dose-response trends in tumor incidence, the same procedures were repeated after reconfiguring the poly-3 test for negative trends. When the frequency of poly-3 p-values for positive trend computed from all tumors in all 10 bioassays in which at least two tumors occurred are considered there is an excess of large p-values (close to 1.0) compared to small p-values (close to 0.0). Since the version of the poly-3 trend test applied is a one-sided test for a positive trend, p-values close to 1.0 would translate into p-values near 0.0 for one-sided trend tests for anti-carcinogenicity. Results of tests for a dose-related decrease in survival in each study show that in none of the bioassays analyzed this test was statistically significant. Moreover, 4 of the datasets had p-values in excess of 0.95 which indicates a significant positive trend in survival with increasing dose. The most significant poly-3 trend in all 10 bioassays was found in the Atkinson *et al.* (1993) study for hemangiosarcoma in male mice with a p-value of 0.0013. The actual significance of this smallest p-value, which is the false positive rate for the min-test, was 0.26 based on the primary analysis, rather than the naive value of 0.0013. This means that 26 % of the randomizations of the 10 datasets gave a smallest p-value less than or equal to the smallest p-value obtained from the original data. Besides, the incidence in hemangiosarcomas (8 %) remained within the historical control range and no such tumors were identified in another mouse study at a dose level nearly 5 times of that used in the Atkinson *et al.* (1993) study. Overall, these findings suggest that, after accounting for the

number of statistical tests performed, there was no clear evidence of a positive dose-related trend in tumor occurrence. The 01-test for a negative trend was highly significant with a p-value of 0.002. These findings suggest stronger evidence for negative rather than positive dose-response trends in tumor occurrence. In all 10 bioassays investigated, the analysis made in this paper identified 24 tumors that exhibited a poly-3 positive trend with a p-value of less than or equal to 0.05. Nevertheless, after accounting for the multitude of statistical tests this analysis did not find that number statistically significant ($p = 0.08$). The statistical analysis of 10 glyphosate bioassays presented in this paper found no strong statistical evidence that glyphosate is carcinogenic. This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because state-of-the-art statistical methods were employed to a selected set of cancer bioassays to demonstrate false-positive probabilities.

Reliability criteria for *in vivo* toxicology studies

Publication: Crump *et al*., 2020.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N.A.	
Study performed according to GLP	N.A.	
Study completely described and conducted following scientifically acceptable standards	Y	Statistical re-analysis of 10 selected bioassays for which individual animal data are available.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Provided in the original bioassays.
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	Provided in the original bioassays.
Test conditions clearly and completely described	Y	Provided in the original bioassays.
Route and mode of administration described	Y	Oral *via* the diet.
Dose levels reported	Y	Provided in the original assays. The maximum doses reported are 5,873 mg/kg bw/day and 4,841 mg/kg bw/day in female and male mice, respectively.
Number of animals used per dose level reported	Y	50 – 64 animals per dose group.
Method of analysis described for analysis test media	N	Should be provided in original bioassays.
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	N.A.	Statistical re-analysis of all tumor sites.
Statistical methods described	Y	Application of a multi-response permutation procedure providing valid false-positive probabilities.
Historical control data of the laboratory reported	Y	For some tumors.
Dose-effect relationship 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 state-of-the-art statistical methods were employed to a selected set of cancer bioassays to demonstrate false-positive probabilities.		

1. Information on the study

Data point:	CA 5.6
Report author	Ganesan S. *et al*.
Report year	2020
Report title	Absence of glyphosate-induced effects on ovarian folliculogenesis and steroidogenesis
Document No	Reproductive Toxicology, (2020) 96, 156-164 DOI: 10.1016/j.reprotox.2020.06.011
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

In vivo study on post natal day 42, glyphosate administered to C57BL/6 J female mice at 0 or 2 mkd (10 mice/dose). Cyclicity, follicle number, circulating ovarian steroid hormone levels and ovarian intracellular signaling parameters were tested in adult female mice during 5 or 10 weeks. Glyphosate exposure for five or ten weeks did not affect the ovarian and endocrine endpoints examined.

The article is classified as reliable with restrictions for the following reasons: only 1 dose tested (no dose relationship can be evaluated), purity of glyphosate is not clear, method of analysis for analysis test media & no validation of the analytical method was described, no GLP status stated, no OECD guideline followed. Although no HCD were available in order to compare with the equivalent concurrent controls and test groups results, the results were negative and therefore did not require HCD to interpret or provide context to any findings.

Reliability Criteria: *In Vivo* Toxicology Studies		
Publication: Ganesan S. *et al*. 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	Not stated
Study completely described and conducted following scientifically acceptable standards	Y	Use of control group, 10 female mice/group, 1 dose + 1 negative control, statistical analysis performed, no randomisation of animals mentioned, no HCD
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate No Purity, no storage condition stated
Only glyphosate acid or one of its salts is the tested substance	N	Glyphosate
AMPA is the tested substance	N	
Study		

Reliability Criteria: *In Vivo* Toxicology Studies		
Publication: Ganesan S. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Test species clearly and completely described	Y	C57BL/6 J mice, age, origin stated but initial BW not included
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Per os
Dose levels reported	Y	0 and 2 mg/kg/d; 5 days/week for 5 and 10 weeks
Number of animals used per dose level reported	Y	10 mice/group 5 weeks (n = 10 control; n = 10 GLY) and 10 weeks (n = 10 control; n = 10 GLY)
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	N	Only 1 dose tested
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	only 1 dose tested (no dose relationship can be evaluated), purity of glyphosate is not clear, method of analysis for analysis test media & no validation of the analytical method was described, no GLP status stated, no OECD guideline followed. Although no HCD were available in order to compare with the equivalent concurrent controls and test groups results, the results were negative and therefore did not require HCD to interpret or provide context to any findings.
Not reliable	N	

1. Information on the study

Data point:	CA 5.8.3
Report author	Gastiazoro M.P. *et al.*
Report year	2020
Report title	Glyphosate induces epithelial mesenchymal transition-related changes in human endometrial Ishikawa cells via estrogen receptor pathway
Document No	Molecular and cellular endocrinology, (2020), Vol. 510, Art. No. 110841
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes / Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

Ishikawa endometrial cancer cells were treated with glyphosate at 0.2 µM and 2 µM. Glyphosate caused cell migration, invasion ability and down regulated E-cadherin mRNA expression. 17ß-estradiol, which was included as a positive control caused similar epithelial mesenchymal transition related changes, while treatment with fulvestrant (estrogen receptor antagonist) reversed the effects caused by glyphosate. The findings suggest that glyphosate has the ability to trigger the estrogen receptor-dependant pathway.

The relevance to human health risk assessment of this unvalidated *in vitro* research model in an immortal adenocarcinoma cell line containing estrogen and progesterone receptors is not clear. The results contradict a number of higher tier studies conducted across a variety of test systems,

The article is classified as reliable with restrictions for the following reason : glyphosate was tested at two different concentrations only, no test guideline was used and no historical control data were provided in order to compare with the equivalent concurrent controls and test groups results. Further, the study was not performed according to GLP.

Reliability criteria for *in vitro* toxicology studies		
Publication: Gastiazoro M.P. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	Not stated
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate (purity ≤ 100%)
Only glyphosate acid or one of its salts is the tested substance	N	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Ishikawa cells

Reliability criteria for *in vitro* toxicology studies		
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	Not necessary
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.2 µM and 2 µM
Cytotoxicity tests reported	Y	Viability was assessed with tryptan blue dye exclusion assay
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	Y	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	Glyphosate was tested at two different concentrations only, no test guideline was used and no historical control data were provided in order to compare with the equivalent concurrent controls and test groups results. Further, the study was not performed according to GLP.
Not reliable	N	

1. Information on the study

Data point:	CA 5.2.6
Report author	Lindberg T. *et al.*
Report year	2020
Report title	An integrated transcriptomic- and proteomic-based approach to evaluate the human skin sensitization potential of glyphosate and its commercial agrochemical formulations
Document No	Journal of proteomics, (2020) Vol. 217, pp. 103647
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes / Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:
Investigation of molecular mechanisms in the skin sensitization process specifically focusing on DC activation using an integrated transcriptomic- and proteomic approach.
First, Mutz-3-derived cells were exposed to PPD, DMSO, unexposed sample and glyphosate. No cytotoxicity was observed for glyphosate and glyphosate was classified as non-sensitising.
Second, PPD, DMSO, unexposed sample and glyphosate were assembled to protein groups. A clear separation between sensitizers (PPD) and non-sensitizers (unexposed, DMSO, glyphosate) was observed. Data on glyphosate are consistent with other available validated assay results.

The article is classified as reliable with restrictions for the following reason: This is a non-validated test system. The purity and origin of glyphosate is unclear. only 1 dose tested (no dose relationship can be evaluated), no HCD were available in order to compare with the equivalent concurrent controls and test groups results.

Reliability Criteria: *In Vitro* Toxicology Studies		
Publication: Lindberg T. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Not applicable because there is no OECD guideline for this type of test
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	The only information is that glyphosate's input concentration was 500 µM. The purity, origin etc. is unclear.
Only glyphosate acid or one of its salts is the tested substance	N	Glyphosate alone and EU non-representative formulations were tested (summary above is provided only for glyphosate)
AMPA is the tested substance	N	

Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	n.a.	Not applicable as no metabolic activation system used
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Not applicable as only 1 concentration was used
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	This is a non-validated test system. The purity and origin of glyphosate is unclear. Only 1 dose tested (no dose relationship can be evaluated). No HCD were available in order to compare with the equivalent concurrent controls and test groups results.
Not reliable	N	

1. Information on the study

Data point:	CA 5.5/027
Report author	Portier, C.J.
Report year	2020
Report title	A comprehensive analysis of the animal carcinogenicity data for glyphosate from chronic exposure rodent carcinogenicity studies
Document No	Environ Health (2020) Vol. 19, 18. https://doi.org/10.1186/s12940-020-00574-1
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	-/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

Thirteen glyphosate cancer bioassays considered acceptable for this re-analysis were selected from the published literature, the EPA review, the review from the German Institute for Risk Analysis, the JMPR review, and full laboratory reports. For twelve of them full study reports were available. Individual tumour counts for the individual studies were re-analyzed using the exact Cochran-Armitage one-sided linear trend test. Re-analyses were conducted on all primary tumours where there were at least 3 tumours in all of the animals in a sex/species/strain combination. In addition, any tumour where a significant positive trend ($p \leq 0.05$) was found in at least one study was also evaluated in all the other studies of the same sex/species/strain combination, regardless of the number of animals with the tumour. Pairwise comparisons between individual exposed groups and the control group were conducted using Fisher's exact test. To evaluate the consistency of a tumour finding across multiple studies using the same sex-species-strain combinations, logistic regression with individual background responses and dose trends are fit to the pooled data using maximum likelihood estimation. The same methods of analysis were used to evaluate the incidence of non-neoplastic lesions in tissues where tumours were observed. In cases of rare tumours where the increase in incidence didn't reach statistical significance the test proposed by Tarone (1982) was applied using an appropriate historical control group. To summarize the results of the strength-of-evidence analysis, each tumour is placed in any of the following categories: Clear evidence (CE), some evidence (SE), equivocal evidence (EE), and no evidence (NE). The factors used to place tumours into these categories include the analyses of the individual studies, the consistency of the data across studies (pooled analyses), the analyses using historical control data, the analyses of non-neoplastic lesions, and mechanistic evidence with the associated scientific literature. The author's weight-of-evidence analysis indicates that there is clear evidence (CE) that oral exposure to glyphosate *via* the diet produces adrenal cortical carcinoma in the female SD rat, hemangioma in the female mouse (CD-1 and Swiss albino), hemangiosarcoma in the male CD-1 mouse, kidney tumours in the male CD-1 mouse and SD rat, liver adenoma in the male rat (SD and Wistar), malignant lymphoma in the male and female CD-1 mouse, skin basal cell tumour in the male SD rat and skin keratoacanthoma in male rats (SD and Wistar). Some evidence (SE) for a causal relationship was put forth for kidney tumours in the male Swiss albino mouse, mammary tumours in the female Wistar rat, malignant lymphoma in the male and female Swiss albino mouse, pituitary adenoma in the male and the female Wistar rat, and testicular interstitial cell tumours in the male SD rat.

After thorough analysis and considering all factors that are important in the interpretation of cancer studies none of the tumours identified by the author as indicating clear evidence (CE) or some evidence (SE) of carcinogenicity were found relevant for reconsideration under the necessary due diligence of the European AIR5 review of glyphosate. Most of the tumours selected by the author were previously dismissed by the EU experts as not relevant even before the last review of glyphosate

in 2017, and the applicant believes that there is no solid toxicological evidence for glyphosate exposure related carcinogenicity in the mouse and the rat that warrants any science-based concerns for human health. The discussion of each of the suspect tumours is given below.

Clear evidence (CE) for carcinogenicity:

Adrenal cortical carcinoma in the female SD rat (Stout and Ruecker study):

The tumour incidences were 0/60, 0/60, 0/60, 3/60 at 0, 113, 457, and 1183 mg/kg bw/day, respectively. This tumour has not been considered treatment related by the authors of the study. There is no dose-related increase in adrenal cortical adenoma (1/60, 3/60, 2/60, 1/60), no dose-related increase in pre-neoplastic lesions and this tumour was not found in the males of the same study or in other rat studies. Therefore, this tumour has been considered not relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hemangioma in female CD-1 mice (Sugimoto study):

In the Sugimoto study hemangiomas were observed in different tissues:

- In liver with an incidence of 0/50, 0/50, 1/50, 1/50;
- In the ovary with an incidence of 0/50, 0/50, 0/50, 1/50;
- In the uterus with an incidence of 0/50, 0/50, 1/50, 2/50;
- In the spleen with an incidence of 0/50, 0/50, 1/50, 0/50;
- In the abdominal cavity with an incidence of 0/8, 0/9, 0/9, 1/9;

At 0, 153.2, 786.8, and 4116 mg/kg bw/day, respectively. Taken together as systemic tumours a significant positive trend is obtained. However, hemangiomas have also been observed in males (liver and testes) but without any dose-response relationship and the highest incidence found was in the control group (1/50). These tumours have not been confirmed in the other carcinogenicity studies in the CD-1 mouse. Moreover, the dose level (4116 mg/kg bw/day) at which the incidence was statistically significantly increased when compared against the control, is more than 4-fold the limit dose for the testing of carcinogens in rodent species. If that dose is ignored there is no significative positive trend. Therefore, this tumour has been considered not relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hemangioma in female Swiss albino mice (Kumar study):

In the re-analysis of the tumour data of the Kumar study by K. Weber (report submitted in 2017) no statistically significant trend was found for systemic neoplasms using the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Hemangiosarcoma in the male CD-1 mouse (Atkinson study):

The tumour incidences were 0/50, 0/50, 0/50, 4/50 (3 in liver and 1 in prostate) at 0, 98, 297, and 988 mg/kg bw/day, respectively. The incidence at the highest dose (8 %) was still within the historical control range of the test laboratory (0-8 %, 300 male mice in 6 studies up to 1993). This tumour was not confirmed in other mouse studies of which one (Knezevich and Hogan study) with a dose level nearly 5-fold that of the Atkinson study (4841 mg/kg bw/day). Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Kidney tumours in the male CD-1 mouse (Takahashi study, as reported by JMPR, 2016):

Renal cell adenoma (3/50) and renal cell carcinoma (1/50) were observed in males at 7470 mg/kg bw/day, but, according to the authors, there was no statistically significant difference with the control group. It is of note that the high dose considered in this study for males is extraordinarily high, more than 7-fold the limit dose for the testing of carcinogens in rodent species. If this dose is ignored there is no significant positive trend. These tumours were re-examined by the original study pathologist in 2012 because the Pesticide Expert Panel of the Food Safety Commission of Japan requested more information on historical control data and association with the non-neoplastic renal findings. After re-examination, the incidences for renal cell adenoma were 1/50, 1/50, and 1/50 at 167.6, 685, and

7470 mg/kg bw/day, showing no dose-response relationship. The incidence for renal cell carcinoma was confirmed to be 1/50 at 7470 mg/kg bw/day. The historical control data for the Takahashi study were not available, but the historical control values described in the re-examination document for renal cell carcinoma were 1/725 (0.13 %) in males and 0/725 (0 %) in females and for renal cell adenoma were 3/564 (0.53 %) in males and 0/564 (0 %) in females. The re-examination report also provides reference data of 0-1.8 % in males and 0 % for all doses in females for renal cell carcinoma, and 0-1.8 % in males and females for renal cell adenoma. The results of the re-examination revealed also that the tubular epithelial cell hypertrophy was localized with an incidence in each treatment group that did not significantly differ from that in the control group. There was no association between the tubular epithelial cell hypertrophy and the development of renal tumours. The renal cell tumours observed in this study are thus not relevant for the human risk assessment of glyphosate because (1) the incidence of renal tumours in males at 7470 mg/kg bw/day did not significantly differ from that in the control group upon re-evaluation; (2) none of the females had neoplastic or non-neoplastic lesions; and (3) the high dose considered in this study for males is more than 7-fold the limit dose for the testing of carcinogens in rodent species. Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Kidney tumours in the male SD rat (Enemoto study):
The incidences of kidney adenoma were 0/76, 0/75, 0/80, 4/78 at 0, 104, 354, and 1127 mg/kg bw/day. An increasing trend in the incidence of adenomas in the kidney was observed in males of the high dose group (animal 193: killed in extremis at week 92, animal 167: found dead at week 94, animal 159: found dead at week 101, and animal 169: killed by design after 104 weeks of treatment) and this incidence was greater than the historical control range referred to in the study report (0-2.9 %). However, according to the authors of this study, the increase observed was not statistically significant. No kidney tumours were found in the females and nearly all male rats at all dose levels suffered from chronic nephropathy (62/76, 63/75, 56/80, 67/78). This tumour in this study was not considered relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hepatocellular adenomas in the male SD rat (Stout and Ruecker study):
The tumour incidences for adenomas were 3/60, 2/60, 3/60, 8/60 and of carcinomas were 3/60, 2/60, 1/60, 2/60 at 0, 89, 362, and 940 mg/kg bw/day, respectively. The incidence of adenomas at the high dose (13.3 %) is still within the historical control range of the test laboratory (1.4-18.3 %). Foci of cellular alteration were observed at all dose levels without any dose-response relationship and there were no signs of hepatocellular hypertrophy, a prerequisite for hepatocellular carcinogenesis. Beside the Brammer study no increase in hepatocellular adenomas was noted in the other rat studies. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Hepatocellular adenomas in the male Wistar rat (Brammer study):
The tumour incidences were 0/64, 2/64, 0/64, 5/64 at 0, 121, 361, 1214 mg/kg bw/day, respectively. The positive trend is significant and the incidence at the high dose is significantly different from the control. However, the incidence at the high dose (7.8 %) is still within the historical control range of the test laboratory (0-11.5 %, 26 studies in 1984-2003). There were no histopathological signs of liver enzyme induction or pre-neoplastic lesions. The high dose animals in this study survived longer when compared to the other groups. This may also influence the spontaneous tumour rate. Beside the Stout and Ruecker study no increase in hepatocellular adenomas was noted in the other rat studies. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the male CD-1 mouse (Sugimoto study):
The tumour incidences were 2/50, 2/50, 0/50, 6/50 at 0, 165, 838, 4348 mg/kg bw/day, respectively. The positive trend is significant but the incidence at the high dose is not significantly different from the control. Moreover, the incidence at the high dose (12 %) is still within the historical control range of the test laboratory (3.6-19.2 %, 458 male mice in 12 studies in 1993-1998). The trend has been found significantly positive because of the elevated incidence at a dose level that is over 4-fold the limit dose for carcinogenicity studies in rodents. If this dose is ignored the trend is not positive. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the female CD-1 mouse (Takahashi study):
The tumour incidences were 3/50, 1/50, 4/50, 6/50 at 0, 93.2, 909, and 8690 mg/kg bw/day, respectively. The increased incidence of lymphoma at the high dose was statistically significant in the trend test but not in a pairwise comparison. The trend has been found significantly positive because of the elevated incidence at an extraordinarily high dose level, more than 8-fold the limit dose for carcinogenicity studies in rodents. If this dose is ignored the trend is no longer significant. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Skin basal cell tumour in the male SD rat (Enemoto study):
The tumour incidences were 0/78, 0/75, 0/80, 3/78 for adenoma and 0/78, 0/75, 0/80, 1/78 for carcinoma at 0, 104, 354, and 1127 mg/kg bw/day. No increased incidence of this tumour was observed in the females or other rat studies and may be associated with other skin lesions (follicular hyperkeratosis and/or folliculitis/follicular abscess) observed in this study. Although there is a significant positive trend for the adenomas, the increase in incidence at the high dose level was not considered relevant for the risk assessment of glyphosate by the authors of this study. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat (Stout and Ruecker study):
The tumour incidences were 1/60, 3/60, 4/60, 5/60 at 0, 89, 362, and 940 mg/kg bw/day. Although there is a significant positive trend the incidence at the high dose was not statistically significantly different from the control and considered not related to treatment. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats. Therefore, this tumour was not considered relevant for the risk assessment of glyphosate by the authors of this study. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat (Atkinson study):
The combined incidences of intracutaneous cornifying epithelioma (keratoacanthoma) were 1/50, 2/25, 0/19, 0/21, 5/50 at 0, 11, 112, 320, and 1147 mg/kg bw/day. Although the trend was significant, the incidence at the high dose was not statistically significantly different from the control and considered not related to treatment by the authors of this study. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat (Enemoto study):
The incidences of the tumour were 4/76, 3/75, 0/80, 7/78 at 0, 104, 354, and 1127 mg/kg bw/day. The increased incidence of this skin tumour at the high dose may be associated with other skin lesions (follicular hyperkeratosis and/or folliculitis/follicular abscess) observed in this study. Although there is a significant positive trend for this tumour, the increase in incidence at the high dose level was not statistically significantly different from the control. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats and considered by the authors of this study not relevant for the risk assessment of glyphosate. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male Wistar rat (Wood study):
There were no treatment-related conditions seen in the skin or in subcutaneous tissues, but several spontaneous lesions were observed. Epidermal ulceration and scab formation, inflammatory lesions, abscess formation, focal acanthosis, focal mineralisation, focal dermal thickening, and focal necrosis were seen, occasionally or rarely and without significance. This tumour was not discussed further in the previous EU review of glyphosate.

Some evidence for carcinogenicity (SE)
Kidney tumours in the male Swiss albino mouse (Kumar study):
In the re-analysis of the tumour data of the Kumar study by K. Weber (submitted in 2017) no statistically significant trend was found for systemic neoplasms in the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise

comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Mammary tumour in the female Wistar rat (Wood study):
At interim and terminal sacrifice combined mammary neoplasia was seen in 6 female mice. There were no mammary neoplasms in the control group but carcinomas were seen with incidences of 2/51, 3/51, and 1/51 at 153.2, 786.8, and 4116 mg/kg bw/day, respectively. All neoplasms were adenocarcinomas with the exception of one adenosquamous carcinoma seen in a low dose group animal. No increase in the incidence of these tumours was reported in the females of other rat studies. The authors concluded that there was no effect of treatment upon the incidence of mammary neoplasia in this study. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the male Swiss albino mouse (Kumar study):
In the re-analysis of the tumour data of the Kumar study by K. Weber (2017) no statistically significant trend was found for systemic neoplasms in the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour was not considered as relevant for the assessment of glyphosate.

Pituitary adenomas in the male and the female Wistar rat (Wood study):
Pituitary adenomas were only seen in female mice with incidences of 0/51, 1/51, 0/51, 2/52 at 0, 104.5, 348.6, and 1381.9 mg/kg bw/day. The group distribution was unrelated to treatment. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Testicular interstitial cell tumour in the male SD rat (Lankas study):
The incidences of this tumour were 0/50, 3/50, 1/50, 6/50 at 0, 3.05, 10.30, and 31.49 mg/kg bw/day, respectively. The positive trend is statistically significant and the incidence at the high dose level (12 %) is statistically significantly different from the control and greater that the historical control rate of the test laboratory (3.4-6.6 %). However, there was no dose-response relationship for interstitial cell hyperplasia (1/50, 1/50, 1/50, 0/50). Since the dose range considered in this study (0-31.5 mg/kg bw/day) is approximately at least 30-fold lower than that of all the other studies in rats where no increase of such tumours was found this finding should be considered as spontaneous in nature. Therefore, this tumour was not considered relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because some of the statistical methods employed were not described in sufficient detail. Besides, the results of this study are not in agreement with the findings of Crump *et al*. 2020 in relation to the estimation of false positives and the overall evaluation of the significance of the tumours by the EU regulatory authorities. All the tumours that were identified by the author as providing clear evidence for the carcinogenicity of glyphosate have been previously dismissed in the EU regulatory process.

Reliability criteria for *in vivo* toxicology studies

Publication: Portier, 2020.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N.A.	
Study performed according to GLP	N. A.	Most of the study reports analysed were GLP compliant.

Study completely described and conducted following scientifically acceptable standards	Y	Re-analysis of the tumour data of 13 selected glyphosate cancer bioassays.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of glyphosate used in every cancer bioassay mentioned.
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	N.A.	Described in the test reports of 12 of the selected studies. The data from one study were derived from a JMPR review.
Route and mode of administration described	Y	Oral *via* the diet.
Dose levels reported	Y	Dose range from 71.4 to 8690 mg/kg bw in the mouse and from 3.05 to 4348 mg/kg bw in the rat.
Number of animals used per dose level reported	Y	About 50 per dose group.
Method of analysis described for analysis test media	N.A.	Described in the original test reports.
Validation of the analytical method	N.A.	
Analytical verifications of test media	N.A.	
Complete reporting of effects observed	Y	
Statistical methods described	Y	All statistical methods used in the re-analysis of the tumour data were reported, however sometimes not in sufficient detail.
Historical control data of the laboratory reported	N.A.	
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 some of the statistical methods employed were not described in sufficient detail. Besides, the results of this study are not in agreement with the findings of Crump *et al*. 2020 in relation to the estimation of false positives and the overall evaluation of the significance of the tumours by the EU regulatory authorities. All the tumours that were identified by the author as providing clear evidence for the carcinogenicity of glyphosate have been dismissed in the EU regulatory process.		

1. Information on the study

Data point:	CA 5.8.3
Report author	Xia Y. *et al*.
Report year	2020
Report title	The endoplasmic reticulum stress and related signal pathway mediated the glyphosate-induced testosterone synthesis inhibition in TM3 cells
Document No	Environmental Pollution, (2020) 260, 113949 DOI: 10.1016/j.envpol.2020.113949
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

In vitro study on the effects of glyphosate on testosterone secretion and the role of endoplasmic reticulum stress in the process were investigated in TM3 cells. Results showed that exposure to glyphosate at concentrations below 200 mg/L had no effect on cell viability, while glyphosate at concentrations above 0.5 mg/L could inhibit the testosterone secretion in TM3 cells. Treatment of TM3 cells with glyphosate at 5 mg/L not only reduced the protein levels of testosterone synthase StAR and CYP17A1 but also inhibited testosterone secretion.

The article is classified as reliable with restrictions for the following reason: not enough information on the tested material (purity) was provided, no positive controls were used, and no statistical methods were described. Furthermore, no OECD guidelines were followed, no GLP status was stated, and no historical control data (HCD) were provided to compare the relevance of data. In addition, key literature in disagreement with the authors' findings appear to have been disregarded, suggesting bias within the research and the following publications: Hecker (2011), OECD validation of the H295R steroidogenesis assay with glyphosate; Levine (2007), demonstrating a lack of effect of glyphosate on the StAR protein; US EPA (2015), glyphosate EDSP weight of evidence evaluation; and EFSA (2017), peer review of glyphosate potential endocrine-disrupting properties.

Hecker M et al (2011), The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter-laboratory validation study, Environmental Science and Pollution Research 18(3):503-15

Levine S. L. Et al. (2007), Disrupting mitochondrial function with surfactants inhibits MA-10 Leydig cell steroidogenesis, Cell Biol Toxicol (2007) 23:385-400

US EPA (2015), EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals - EDSP: WEIGHT OF EVIDENCE ANALYSIS OF POTENTIAL INTERACTION WITH THE ESTROGEN, ANDROGEN OR THYROID PATHWAYS - CHEMICAL: GLYPHOSATE

EFSA (2017): Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate, Question number: EFSA-Q-2016-00663

Reliability Criteria: *In Vitro* Toxicology Studies		
Publication: Xia Y. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	Not stated
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate Purity not stated
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	N	TM3 cells (no purity stated)
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	Not necessary
Test concentrations in physiologically acceptable range (<1 mM)	N	0.01 to 200 mg/L
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive controls
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	Not enough information on the tested material (purity), no positive controls were used, no statistical methods were described. Furthermore, no OECD guideline followed, no GLP status was stated. In addtion, no HCD were available in order to compare the relevance of the data. In addition, key literature in disagreement with the authors' findings appear to have been disregarded, suggesting bias within the research and publication: Hecker (2011) OECD validation of the H295R steroidogenesis assay with glyphosate; Levine (2007) demonstrating a lack of effect of glyphosate on the StAR protein; US EPA (2015) glyphosate EDSP weight of evidence evaluation; EFSA (2017) peer review of glyphosate potential endocrine-disrupting properties.

Reliability Criteria: *In Vitro* Toxicology Studies		
Publication: Xia Y. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Not reliable	N	

1. Information on the study

Data point:	CA 5.8.2
Report author	Yahfoufi Z. A. *et al.*
Report year	2020
Report title	Glyphosate Induces Metaphase II Oocyte Deterioration and Embryo Damageby Zinc Depletion and Overproduction of Reactive Oxygen Species
Document No	Toxicology, (2020) Vol. 439, Art. No. 152466
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 quality of metaphase II noncumulus oocytes and embryos from mice were investigated following glyphosate exposure at different concentrations (max 300 μM). The concentrations were in the range of those found in human blood following accidental acute exposure or suicidal attempts.

Results indicate that glyphosate provokes disruption of the microtubule organizing center and chromosomal disorganization at the mid-position of the spindle due to spindle disappearance, and defective chromosomal alignment as well as depletion of intracellular zinc bioavailability and enhancement of reactive oxygen species (ROS) accumulation in the mouse oocytes. In the embryos (not specified the source and the embryonal stage) zinc depletion and accumulation of ROS was also observed in a dose-related manner.

The article is classified as *reliable with restrictions* for the following reason: Not performed according to GLP or an OECD test guideline. No purity of the test substance stated. No information of the source and the embryonal stage of the embryos were provided. There were no concurrent positive control or substances known to deteriorates oocyte quality through disassembly of microtubule organizing centers (like peroxynitrite) or ROS accumulation (like hydrogen peroxide, and hypochlorous acid) or dimercapto-1-propanesulfonic acid (DMPS) for zinc depletion.

Reliability criteria for *in vitro* toxicology studies		
Publication: Yahfoufi Z. A. et al. 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	No GLP or OECD test guideline followed
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	No purity, content, storage conditions
Only glyphosate acid or one of its salts is the tested substance	Unknown	
AMPA is the tested substance	N	
Study		

Reliability criteria for *in vitro* toxicology studies		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	No metabolic activation system present
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Max. concentration 300 µM
Cytotoxicity tests reported	N	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	Not performed according to GLP or an OECD test guideline. No purity of the test substance stated. No appropriate information on the tested embryos was provided. Tested dose correspond to level of glyphosate that may be found in human blood following human accidental acute exposure intoxication. No reference compound were tested in parallel to confirm the appropriateness of the testing procedures and the relevance of the results.
Not reliable	N	

検索期間：2020年1月～6月

区分ａに分類された文献とその理由

環境動態

1. Information on the study

Data point:	CA 7.5
Report author	De Polo, A. *et al.*
Report year	2019
Report title	From the traces in the wells of the urban aqueduct network to the subsequent prohibition of the use of glyphosate: the case of an area of high-intensity wine production in the province of Treviso, Veneto. Original Title: Dai residui nei pozzi della rete acquedottistica urbana al successivo divieto di utilizzo del glifosate: il caso di un'area ad alta intensità vitivinicola in provincia di Treviso, Veneto.
Document No	Igiene e sanità pubblica, (2019) Vol. 75, No. 6, pp. 451-460
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes / Reliable with restrictions

2. Assessment and conclusion

Assessmentand conclusion by applicant:
The article reports concentrations of glyphosate and AMPA found in 12 wells used for drinking water consumption in Northern Italy, situated within a wine growing area. Traces of glyphosate (maximum reached 0.08 µg/L) and AMPA (maximum reached 0.25 µg/L) were detected in 2 wells supplying an urban area. The article is therefore considered as reliable with restrictions. Sampling and analytical methods are not described. Nature of groundwater wells not described, point sources could be possible. No information on precipitation is reported. No description of monitoring sites other than very rough map of area and aqueduct names.

1. Information on the study

Data point:	CA 7.1.4.2
Report author	Gros, P. *et al.*
Report year	2020
Report title	Leaching and degradation of $^{13}C_2$-^{15}N-glyphosate in field lysimeters
Document No	Environmental monitoring and assessment, (2020) Vol. 192, No. 2, pp. 127 DOI 10.1007/s10661-019-8045-4
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 article reports results of a lysimeter experiment with $^{13}C_2$-^{5}N-glyphosate in Germany. Besides analysis of lysimeter leachate, also soil and plant (maize) samples were analyzed. Although, the methods and results are well described, no endpoint can be derived due to some deviations from the relevant guideline (OECD 22). For example, it is not clear whether an undisturbed soil monolith has been used, and the origin and storage of soil is not reported. Amounts of precipitation are not reported in sufficient detail (only for 2 months) and amounts of leachate are only given as overall sum and weekly averages. glyphosate, the sensitivity of the analytical method is not reported (AMPA was analyzed only qualitatively), and the stability of analytes in leachates and extracts during frozen storage was not shown. Results of leachate analysis were not reported in µg/L (only as $\delta^{13}C$ and $\delta^{15}N$), and results of soil analysis were only given in % of initial concentration.

The article is therefore considered as reliable with restrictions.

検索期間：2020年1月～6月

区分ａに分類された文献とその理由

生活環境動植物及び家畜に対する毒性

1. Information on the study

Data point:	CA 8.6.2
Report author	Rogacz D. *et al.*
Report year	2020
Report title	Ecotoxicological effects of new C-substituted derivatives of *N*-phosphonomethylglycine (glyphosate) and their preliminary evaluation towards herbicidal application in agriculture.
Document No	Ecotoxicology and environmental safety, (2020) Vol. 194, Art. No. 110331.
Guidelines followed in study	OECD 208 (for NTTPs)
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes / Reliable

2. Assessment and conclusion

Assessment and conclusion by applicant:

The study investigated the effects of glyphosate on the seedling emergence and growth of non-target terrestrial plants (oat and radish) based on OECD 208 guideline. Plants were exposed to glyphosate mixed into sandy soil at 5 concentrations between 100 and 100 mg a.s./kg of soil dry weight with 3 replicates each. There were 20 seeds of each plant species per test concentration sown into the soil. Evaluations were based on fresh mass, root length and shoot height of plants after 14 days of exposure.

The test design was adequately described, but the application of the test item into the soil is not specified. The seedling emergence was acceptable as recommended in the guideline (100 and 95 % in the control for oat and radish, respectively). However, the phytotoxic effects and the survival of the control plants during the study is not reported.
Reliable endpoints for the risk assessment of NTTPs can be obtained for glyphosate: EC_{50} value of 373.7 mg a.s./kg s.d.w. for oat based on shoot height and an EC_{50} value of 357.8 mg a.s./kg s.d.w. for radish based on shoot height.

The article is classified as reliable for NTTPs.

1. Information on the study

Data point:	CA 8.1.4
Report author	Turhan D. Ö *et al.*
Report year	2020
Report title	Developmental and lethal effects of glyphosate and a glyphosate-based product on *Xenopus laevis* embryos and tadpoles
Document No	Bulletin of Environmental Contamination and Toxicology, (2020) Vol. 104, No. 2, pp. 173-179
Guidelines followed in study	ASTM (2003) American Society for Testing and Materials, Standard guide for conducting the Frog Embryo Teragonesis Assay-*Xenopus* (FETAX), E1439-98
Deviations from current test guideline	Jelly coats should be removed from developing embryos mid blastula stage embryos – ca. stage 8, prior to 96 hour exposure well plates. The removal is achieved using 2% cysteine solution followed by rinsing. No reference is provided in the article to see whether this step was completed (it is a relevant as it informs on ion-exchange and impacts from concentrated solution / osmotic and diffuse pressures on the embryos in the increasing concentrations of test media.) Test guideline followed for the late development stage 46 (96 hr test) exposure test was not stated. The FETAX assay runs until 80-90% of the control tadpoles reach developmental stage 47. This cannot be confirmed. Water quality at test start appears to be within specification, although after 96 hrs now water quality data appeared to have been determined.
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes / Reliable with restrictions

2. Assessment and conclusion

Assessment and conclusion by applicant:

Effects of pure glyphosate and a glyphosate-based product Roundup® Star (containing glyphosate in a form of a potassium salt and including 6% surfactant as ethoxylated alkylamine based, were evaluated comparatively using two embryonic development stages of the amphibian *Xenopus laevis* as model system. As the glyphosate-based product Roundup® Star is not the representative formulation for the European renewal of glyphosate, the summary only provides information for pure glyphosate.

However, this publication confirms a general trend that toxic effects caused by glyphosate-based products, compared to pure glyphosate, are increased mainly due to additives present in glyphosate formulations and that it may be a result of synergistic effects between glyphosate and adjuvant in the formulations.

In this study, no lethality >17 % or developmental effects (growth inhibition) were observed in embryos or tadpoles with pure glyphosate at any glyphosate concentration tested (282-500 mg/L for stage 8 embryos) and (250-403 mg/L stage 46 tadpoles)
In addition, no effect was observed with regards to enzymatic activity of stage 46 tadpoles at any glyphosate concentration tested (50-250 mg/L).

The article is classified as reliable with restrictions for the following reason : The specific purity of the test item was not reported. No OECD guidance has been followed. The American Society for Testing and Materials, Standard guide for conducting the Frog Embryo Teragonesis Assay-Xenopus (FETAX test), E1439-98 has been followed with some deviations to the recognised approach (see deviations above). The FETAX assay is a developmental toxicity screening test, which for the most part has been superseded by amphibian metamorphosis and developmental toxicity assays using *Xenopus laevis* (OECD 231 and OECD 241). Studies performed according to both of these recognised test guidelines were submitted with the Annex I dossier (M-CA Section 8.2.3/002 and M-CA Section 8.2.3/003). Whilst the FETAX assay is not directly recognised at the EU level, elements of the FETAX assay are considered in the conduct of the OECD 231 test guideline.
Control mortalities were not reported (only LC_{50} final results). Analytical verifications of the concentrations in the test medium were reported only before starting the test, but exposure medium was changed every 24 h to maintain the desired concentrations.