WCSR Advice 2016-07

SCIENTIFIC COMMITTEE REACH (WCSR)

ADVICE ON THE PROPOSED HARMONIZED CLASSIFICATION AND LABELLING OF GLYPHOSATE (CARCINOGENICITY AND MUTAGENICITY ENDPOINTS)

WCSR Advice 2016-07

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Context

The classification and labelling of certain hazardous chemicals must be harmonised to ensure adequate risk management throughout the European Union.

Member States, manufacturers, importers and downstream users may propose a harmonised classification and labelling of a substance. Member States can also propose a revision of an existing harmonisation.

The harmonised classification and labelling process (CLH) includes a period of public consultation that lasts 45 days.

Anyone can comment on a proposed harmonisation. Those most likely to be interested are companies, organisations representing industry or civil society, as well as individual experts.

Germany submitted such proposal for harmonised classification and labelling of the substance Glyphosate (EC 213-997-4) to ECHA on 17 March 2016. The CLH report was published on the ECHA website on 2/6/2016 for public consultation with deadline for commenting 18/7/2016.

The outcome of this assessment can be used by the Belgian Competent Authority for commenting the public consultation.

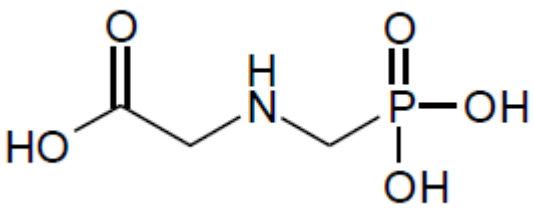
Substance Identity

Public Name: Glyphosate

EC Number(s): 213-997-4

CAS Number(s): 1071-83-6

Structural formula:



CONCERN

A CLH dossier is published on the ECHA website (2/6/2016) for public consultation proposing a harmonized classification and labelling for Glyphosate in the hazard class STOT RE, Serious eye damage/eye irritation and hazardous to the aquatic environment :

Eye Dam. 1, H318

STOT RE 2, H373

Aquatic Chronic 2, H411

ANALYSIS OF AVAILABLE INFORMATION

CLH report Glyphosate: section 4.8 and 4.9

EFSA report: Conclusions on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015; 13(11): 4302 – section Mammalian toxicity

Final addendum to the Renewal Assessment Report Glyphosate, CA RMS Germany, October 2015. Volume 1: 2.6.5 Summary of genotoxicity, 2.6.6 Summary of long-term toxicity and carcinogenicity. Volume 3: B.6.4 Genotoxicity, B.6.5 Long-term toxicity and carcinogenicity. RAR addendum: Does glyphosate cause cancer (April 2015). RAR addendum 1 to RAR (August 2015): Assessment of IARC Monographs vol 112 (215) Glyphosate.

CONCLUSIONS

1 GERM CELL MUTAGENICITY (MUTAGENICITY)

1.1 Non-human information

Only data on the active substance and standard test systems data were considered by the rapporteur for C&L purpose. In addition, there is a lot of published data with glyphosate containing formulations available (which were also assessed by IARC). However, it is likely that some of the results (positive or equivocal) in these studies (including non-standard indicator tests) were due to the co-formulates (e.g. surfactants) and not to the active substance.

As cited in the CLH report, most of the parent substance glyphosate is eliminated unchanged and only a small amount (in most studies less than 1%) is transformed to

aminomethylphosphonic acid (AMPA) in animals. Reliable kinetic data obtained in humans are not available for glyphosate, but there are indications that biotransformation of ingested glyphosate to AMPA is very limited also in man. AMPA has been broadly investigated for many toxicological endpoints and exhibited similar or lower toxicity than glyphosate and was found to be devoid of genotoxic potential.

1.1.1 In vitro data

Negative results

Gene/point mutations in bacteria: Sixteen valid Ames test studies with the active substance glyphosate were assessed. The results were consistently and unequivocally negative.

Mammalian cells, point mutation tests: 2 mouse lymphoma assays, 1 HGPRT test. Negative for genotoxicity.

Mammalian cells, clastogenicity tests: human lymphocytes (2), CHL cells (2): negative, no evidence of clastogenicity.

1 UDS assay in rat hepatocytes, 1 Rec assay in B. subtilis: no impact on DNA damage and repair.

Positive results

Higher rates of SCE (sister chromatid exchange) and chromosomal aberrations in human and bovine lymphocytes at maximum concentrations of 51 or 170 µM (Lioi et al., 1988a and 1988b). Moreover, evidence of increased SCE in human lymphocytes at dose levels of 1mg/mL up to 6 mg/mL was found (Bolognesi et al., 1997). Increased micronucleus formation in human lymphocytes at cytotoxic concentrations of 580 µg/mL (approx. 3.43 mM) when S9 mix had been added (Mladinic et al.,2009a) . A significant and dose related increase in micronucleus frequency in human cells of buccal origin was observed at 15 and 20 µg/mL (Koller et al., 2012). 5 comet assays were positive (Monroy et al., 2005; Mañas et al., 2009; Mladinic et al., 2009b; Koller et al., 2012; Alvarez-Moya et al., 2014), but the findings in high dose range (> 3 mM) were always accompanied with cytotoxicity. No clear dose response relationship is observed at lower dose ranges.

All standard assays performed under GLP conditions were negative (bacterial assays, mammalian cell gene mutation assays). The majority of the chromosomal aberration and micronucleus tests were negative. Positive results were found in indicator tests for induction of SCE and DNA strand breaks but at cytotoxic concentrations but negative for induction of DNA repair.

1.1.2 In vivo data

Germ cells: 2 dominant lethal tests (rat, mouse): negative

Chromosomal aberration studies and micronucleus assays (7 of 8 negative, 1 weak positive but at very high concentration, and the cytogenetic study conducted at the same laboratory with nearly the same conditions and mouse strain was negative) in bone marrow, mice and rats, oral and i.p. administration: Overall conclusion: not clastogenic *in vivo*.

Published studies: other methods with methodological limitations (comet assay, alkalin elution assay, SCE) gave equivocal results. In most of these studies, relatively low dose levels were employed and the intraperitoneal route was used which does not properly reflect the human exposure.

Using the weight of evidence approach, it was concluded that there is no *in vivo* genotoxicity and mutagenicity potential for the active substance glyphosate.

1.2 Human information

The epidemiological data available concerns the use of formulations containing glyphosate but not the active substance itself, and meanwhile the study persons are exposed to other plant protection products. The data are hardly interpretable and can only be used with caution.

1.3 Other relevant information

1.4 Summary and discussion of mutagenicity

There is a predominance of negative results in the well–conducted core assays as the *in vitro* bacterial reversion and the *in vivo* mammalian micronucleus and chromosomal aberration assays. In addition negative results were obtained in the in vitro gene mutation and the majority of results for chromosomal effect assays in mammalian cells.

Equivocal/contradictory results were obtained in published studies with methodological limitations. Nevertheless, with the weight of evidence approach: the active substance, glyphosate, is considered not mutagenic below toxic dose levels. Agreed.

1.5 Comparison with criteria

The active substance, glyphosate, is considered not mutagenic following the criteria for classification for germ cell mutagens as given in the CLP regulation.

Cat 1A: There is no positive evidence available from epidemiological studies. Human data is considered inadequate/inconclusive.

Cat 1B and Cat 2: There is no sufficient evidence in reliable *in vivo* and in *vitro* test systems/studies. On the contrary, there is a predominance of negative results in the reliable

core assays and the majority of the other well-conducted assays. Positive results were mostly observed in indicator tests.
Agreed.

1.6 Conclusions on classification and labelling
According to the CLP criteria, no hazard classification for mutagenicity is warranted for the active substance glyphosate. We agree with the German CA.

2 CARCINOGENICITY

2.1. Non-human information
New toxicological studies were submitted and in addition, a large number of scientific publications were considered in the re-evaluation and for the CLH dossier. We agree with the CA/RMS Germany that for C&L purpose only data on the active substance were considered and a weight of evidence approach was used.

2.1.1 Carcinogenicity: oral
Available studies:
Rats: 9 unpublished long-term feeding studies with glyphosate (>96%) of which 6 performed in compliance with OECD 453. The other 3 flawed by serious deficiencies:
Bhide, 1997*; ASB2012-11489: poor study with many serious reporting deficiencies including lacking information on test material, surprisingly low spontaneous tumour incidences in the controls but the number of animals undergoing histopathology was also low; study rejected for EU risk assessment process;
Lankas, 1981; TOX2000-595 and TOX2000-1997: study flawed by serious reporting deficiencies and employment of too low dose levels far below an MTD, not acceptable according to current standards but previously often used for regulatory purposes;
Calandra, 1974 deficient IBT study, not guideline-compliant, dose levels much too low for meaningful evaluation, not used for any regulatory assessment during the last decades

2 published studies (1 flawed): 1 with glyphosate salt, 1 with a formulation.

Overall NOAEL $_{\text{long-term}}$ = 100 mg/kg bw/d; Overall LOAEL $_{\text{long-term}}$ = 350 mg/kg bw/d, based on ↓bw, ↓bw gain, ↑liver weight, ↑salivary gland weight, ↑AP, ↓urine

pH, histopathological salivary gland changes, cataract, stomach mucosa irritation or caecum distention.

Mice: 5 unpublished long-term feeding studies with glyphosate (>95%) in compliance with OECD 453. 2 older long-term studies, which do not comply with current standards. No increase in any tumour type had been reported in both of them, but the top dose level was 300 ppm and, thus, much too low for meaningful evaluation.

1 published study on skin promotion.

Overall NOAEL $_{\text{long-term}}$ = 150 mg/kg bw/d; Overall LOAEL $_{\text{long-term}}$ = 800 mg/kg bw/d, based on non-neoplastic effects as those in rats but accompanied by liver pathology and epithelial hyperplasia of the bladder.

Statistical re-evaluations were done according to OECD guidelines 2012, 2002: Fishers exact test (pair-wise) and Cochran-Armitage test (trend) because in the original reports a pair-wise comparison was mainly used and IARC used trend test on the data resulting in a different outcome. It has to be mentioned that the outcome of IARC was based on less studies as they evaluated less studies (2 rat: Stout and Ruecker (1990, TOX9300244), Lankas (1981, TOX2000-595, TOX2000-1997). and 2 mice studies: Knezevich & Hogan (1983, TOX9552381), Atkinson et al. (1993, TOX9552382)).
As there is an unusual large volume of experimental animal data, a weight of evidence approach was used by the CA/RMS Germany: agreed.

Rats
Pancreatic islet cell adenoma
These tumours were observed in 2 studies (Stout and Ruecker (1990, TOX9300244) or Lankas (1981, TOX2000-595, TOX2000-1997)) of which one is now considered insufficient due to the very low doses employed and because of reporting deficiencies. However no statistical significant increase could be found re-evaluating the incidences with the 2 approaches (pair-wise, trend) in 1 study for the higher dose, only for the low dose a significant increase was found, in the other study a significant increase number of adenomas and combined adenomas +carcinomas for the male low dose group and in addition a significantly positive trend for carcinomas in males was found. In conclusion, a clear dose response was missing. No tumours were found in female rats. No such tumours were observed in all the other long-term/carcinogenicity studies performed in rats.

Hepatocellular adenoma and carcinoma

In 1 study (Stout and Ruecker (1990, TOX9300244) a statistically positive trend was observed for adenomas and no positive trend for adenoma and carcinoma combined. There was no significant difference to the incidence in the control group found for the respective treatment groups. No pre-neoplastic findings were observed. No liver tumours were observed in all the other long-term/carcinogenicity studies in 2 different rat strains.

Thyroid C-cell adenoma

In 1 study (Stout and Ruecker (1990, TOX9300244) a statistically significant positive trend was found in female rats for thyroid V-cell adenomas. However, the thyroid is not a target organ, nor was an increase in pre-neoplastic histopathological lesions observed in any of the long-term/carcinogenicity studies in rats.

Interstitial cell tumours of the testes

These tumours were observed in 1 study (Lankas (1981, TOX2000-595, TOX2000-1997), difference being statistically significant for the high dose group (pair-wise), but without clear dose response. These tumours were not observed in all the other long-term/carcinogenicity studies.

Conclusion in rats :

The overall conclusion can be drawn that glyphosate was not carcinogenic to the rat. The occasional increases in few different tumour types (pancreas, liver, thyroid, and testes) were observed in two older studies. These findings were not confirmed in five more recent, guideline-compliant studies.

Mice

Renal adenoma and carcinoma in males

Re-evaluation of the data showed that in 1 study (Knezevich & Hogan (1983, TOX9552381)) where these tumours were observed in male CD-1 mice, a positive trend was confirmed as IARC already showed with their analysis (Dose: 157-4841 mg/kg bw/day). The incidences of renal tubule tumours in males of the 4 CD-1 mice studies were put next to each other, showing an increase in renal tumour incidence over the overall control in the 2 studies exposed to extreme high dose levels (> 4000 mg/kg bw/d), clearly above the MTD and above the limit dose for carcinogenicity testing. The rare tumours were also observed in some control animals. No dose-response was observed. No increase in tumours was found in female mice.

Haemangiosarcoma in males

The re-evaluation of the studies showed that in 2 studies with CD-1 mice a statistically positive trend for haemangiosarcoma was observed. However, putting the data next to each other, no dose response could be found. No increase in tumours was found in female mice.

Malignant lymphoma

Malignant lymphoma is one of the most common spontaneously occurring neoplasms in mice. Statistical re-evaluation was done for all studies, and the historical control data requested and (re-) evaluated for all studies and mouse strains. In Swiss mice (a test strain prone to developing lymphoreticular tumours), the significance depended on the test used, and the control data were very high. Nevertheless a treatment-related effect in this study cannot be completely excluded. In CD-1 mice, after re-analysis, statistical significant increases with dose were seen for male mice in 2 studies (trend test), but the incidence in the control mice were very low compared to the historical data. In another study with CD-1 mice, no increase was found. For CD-1 mice (4 studies), the dose levels versus the malignant lymphoma incidence was checked and put together: no consistent dose response could be observed. In conclusion, there is limited evidence of a carcinogenic potential at a high dose. in mice of a susceptible strain. No evidence of a similar effect was found in female mice in the other studies.

When the incidences of the 3 tumour types were put together for the 4 CD-1 mice studies with regard to dose response, it becomes clear that all these tumours were present over the whole dose spectrum including the control groups. No consistent increase could be observed. In addition, taken the historical control data into account, all top dose incidences were below the maxima. In addition the Renewal Assessment Report mentions that "the quality and the regulatory value of the historical control data is very much compromised by the fact that the sexes were not considered separately". (Wood, 2009)

Conclusion in mice:

In Table 42, incidences of the three tumour types under discussion in male CD-1 mice in the four glyphosate studies are summarised with regard to dose response. The highest incidences were observed in groups receiving very high doses of glyphosate, i.e., 4841 mg/kg bw/day in case of renal tumours, 1000 and 4348 mg/kg bw/day in case of malignant lymphoma and 1000 mg/kg bw/day with regard to haemangiosarcoma. These dose levels were at or far above the recommended limit for testing of 1000 mg/kg bw/day. It is noteworthy that no similar or stronger

increase of the latter two tumour types was seen in concurrent studies in which similar or even higher doses were administered. Concerning renal tumours, it should be acknowledged that in fact 3/50 animals were affected at a dose level of 4841 mg/kg bw/day but the number of cases in untreated controls or at a dose level of ca 100 mg/kg bw was 2/50 in another study suggesting that this tumour, even if rare, is not uncommon in male CD-1 mice. To conclude, over a wide dose range, there is no evidence of a consistent increase in any tumour type in male CD-1 mice.

Table 42: Summary of selected tumour incidences in male CD-1 mice from four studies with glyphosate and historical control data.

Dose (mg/kg bw per day)	***HC, Maximum % found***	**0**	**0**	**0**	**0**	**71**	**100**	**157**	**165**	**234**	**300**	**810**	**814**	**838**	**1000**	**4348**	**4841**
Study		A	B	C	D	D	B	A	C	D	B	D	A	C	B	C	A
Study duration (months)		24	24	18	18	18	24	24	18	18	24	18	24	18	24	18	24
Survival		20/50	26/50	26/50	39/51	41/51	25/50	16/50	34/50	39/51	29/50	35/51	17/50	27/50	25/50	29/50	26/50
Renal tumours#	*4 (adenoma) 2 (carcinoma)*	1/49	2/50	0/50	0/51	0/51	2/50	0/49	0/50	0/51	0/50	0/51	1/50	0/50	0/50	2/50	3/50
Malignant lymphoma*	*21.7*	2/48	4/50	2/50	0/51	1/51	2/50	5/49	2/50	2/51	1/50	5/51	4/50	0/50	6/50	6/50	2/49
Haemangiosarcoma**	*12.0*	0/48	0/50	0/50	2/51	1/51	0/50	0/49	0/50	2/51	0/50	1/51	1/50	0/50	4/50	2/50	0/49

Study: A = Knezevich and Hogan (1983, TOX9552381), PWG re-evaluation; B = Atkinson et al. (1993, TOX9552382); C = Sugimoto (1997, ASB2012-11493); D = Wood et al. (2009, ASB2012-11492).

Renal tumours: combined incidence of adenoma and carcinoma given for individual studies.

* Study A: Malign lymphoblastic tumours (3 categories) instead of malignant lymphoma which was not mentioned as a pathological entity.

** Whole body/multiple organ.
Highlighted in grey – dosage exceeded the OECD-recommended limit dose of 1000 mg/kg bw/day and the MTD.
HC: Historical control data for Crl:CD-1 (ICR) mice from Charles River Laboratories (Giknis and Clifford, 2005, ASB2007-5200)

2.1.2 Carcinogenicity: inhalation

No studies

2.1.3 Carcinogenicity: other routes

No studies

2.1.4 Carcinogenicity: dermal

No studies

2.2 Human information

The number of adequate epidemiological studies is limited. Since, it is not possible to distinguish between effects of the active substance glyphosate and its co-formulants since humans are always exposed to plant protection products and their residues but hardly ever to the active substance alone.

IARC: 12 cohort studies, 16+6 case-control studies, meta-analyses. IARC classified glyphosate from studies in humans in the category 'limited evidence of carcinogenicity.

However, in the human studies the exposed study persons were always exposed to glyphosate-based formulations (containing surfactants) and mostly meanwhile also to other plant protective products. For C&L according to EU CLP regulation we should focus on the active substance glyphosate.
Study results are inconsistent, in most studies several products and/or formulations are studied. Most studies are of insufficient quality, statistical power. According to the RMS/CA Germany no consistent positive associations were observed, with the most powerful study showing no effect. Agreed.

2.3 Other relevant information
Not applicable

2.4 Summary and discussion of carcinogenicity
A weight of evidence approach was used on the data of 7 long-term and carcinogenicity studies in rats and 5 carcinogenicity studies in mice. Statistical re-evaluation (pair-wise and trend test) was done on the tumour incidences and the biological significance of the findings discussed.
Rat studies: No consistency could be found for any of the tumour types.
Mice studies: The evidence of increases in the 3 types of tumours were only seen in males, but there was no consistency between the studies. The higher incidences were observed at doses above limit dose of the OECD guidelines for testing, and depending on the statistical analysis used (contradictory results depending on the test used). The incidences felt within the historical control data range. There was no clear dose response when putting all the available data together.

2.5 Comparison with criteria
Human data is considered inadequate/inconclusive and therefore insufficient to classify glyphosate as Carc. 1A.
As there is an unusual huge dataset available with several valid long-term/carcinogenicity studies in different rat and mice strains, a weight of evidence approach was used considering all data together, considering the consistency of the neoplastic findings, the biological significance. In rats, the tumours were only occasionally seen, without clear dose response, and could not been found back in the other studies of the huge data set of valid studies. In mice, the situation is more complex. For malignant lymphoma, a common tumour in mice, there is only very limited evidence of a carcinogenic potential at a high dose (not representative for human exposure and much higher than the proposed ADI) in mice of a susceptible strain (Swiss). No evidence of a similar effect was found in female mice in the other studies (CD-1 mice).

When the incidences of the 3 tumour types (malignant lymphoma, renal adenoma and carcinoma in males, haemangiosarcoma in males) were put together for the 4 CD-1 mice studies with regard to dose response, it becomes clear that all these tumours were present over the whole dose spectrum including the control groups. No consistent increase could be observed. In addition, taken the historical control data into account, all top dose incidences were below the maxima. Taken a weight of evidence approach because of the enormous reliable data set of long-term/carcinogenicity studies in experimental animals, according to the EU CLP criteria for C&L the evidence is not sufficient to meet the criteria for classification as Carc. 1B or Carc. 2.
Agreed.

2.6 Conclusions on classification and labelling
It cannot be denied that tumours were observed in mice, however there was no consistency (dose-response, strain, sex, incidence within historical control data range, statistical evaluation) in the unusual rich valid study material available. The neoplastic findings (malignant lymphoma) were observed in mice at very high doses (above limit dose for testing) not representative for human exposure and in addition no mutagenic potential was observed. In conclusion, the biological significance for humans is far from decisive.
According to the CLP regulation: the evidence does not support classification. Agreed with the CA/RMS Germany.

ADVICE

Agreeing with the CA/RMS DE and the expert panel that also went to the huge review process, that the active substance glyphosate is unlikely to be mutagenic or to pose a carcinogenic threat to humans and is not proposed to be classified as such under EU regulations.

Mutagenicity
Cat 1A: There is no positive evidence available from epidemiological studies. Human data is considered inadequate/inconclusive.
Cat 1B and Cat 2: There is no sufficient evidence in reliable *in vivo* and in *vitro* test systems/studies. On the contrary, there is a predominance of negative results in the reliable core assays and the majority of the other well-conducted assays. Positive results were mostly observed in indicator tests.

Carcinogenicity

Human data is considered inadequate/inconclusive and therefore insufficient to classify glyphosate as Carc. 1A.
It cannot be denied that tumours were observed in mice, however there was no consistency (dose-response, strain, sex, incidence within historical control data range, statistical evaluation) in the unusual rich valid study material available. The neoplastic findings (malignant lymphoma) were observed in mice at very high doses (limit dose for testing) not representative for human exposure and in addition no mutagenic potential was observed. In conclusion, the biological significance for humans is far from decisive.
Taken a weight of evidence approach because of the enormous reliable data set of long-term/carcinogenicity studies in experimental animals, according to the EU CLP criteria for C&L the evidence is not sufficient to meet the criteria for classification as Carc. 1B or Carc. 2.

MEMBERS OF THE SCIENTIFIC COMMITTEE

The members are :

Willy Baeyens; Johan Bierkens; Marie-Noëlle Blaude; Steven Broekx; Peter Dubruel; Lieve Geerts; Lode Godderis; Walter Hecq; Birgit Mertens; Guy Schroyen; Stefaan Soenen; An Van Nieuwenhuyse; Jeroen Vanoirbeek; Reinhilde Weltens.

CONFLICT OF INTEREST

No member has declared any conflict of interest.

RAPPORTEUR

The Scientific Committee REACH thanks the rapporteur Lode Godderis.

ADOPTION OF THE ADVICE

The Scientific Committee REACH advice was adopted by consensus by written procedure on 15/7/2016.

LEGAL FRAMEWORK OF THE ADVICE

Cooperation agreement of 17 October 2011 between the Federal State, the Flemish Region, the Walloon Region and the Brussels Capital Region concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

Ministerial decree of 8 July 2014 appointing the members of the Scientific Committee REACH established under Article 3, § 3 of the Cooperation Agreement of 17 October 2011 between the Federal State, the Flemish Region, the Walloon Region and the Brussels Capital Region concerning the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH)

Ministerial decree of 2 June 2016 on dismissal and appointment of members of the Scientific Committee REACH

DISCLAIMER

The Scientific Committee REACH reserves, at any time, the right to change this advice when new information and data become available after the publication of this version.

President

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c /o

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