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Attached to this is an official copy of the *Opinion proposing harmonised classification and labelling at EU level of glyphosate (ISO); N-(phosphonomethyl)glycine* issued by the Committee for Risk Assessment (RAC), with the EC Number: 213-997-4 and CAS Number: 1071-83-6, adopted on 15th March 2017, initialed by an authorized Officer, and which I certify was obtained from the official files of ECHA and is authentic, accurate, and complete.

Signature

Tim Bowmer, chairman of the Committee for Risk Assessment (RAC)

Place and Date



15/18

Dewayne Johnson v.
Monsanto Company

Defendant's Exhibit 2320

Case No: CGC-16-550128



**Committee for Risk Assessment
RAC**

Opinion

proposing harmonised classification and labelling
at EU level of

glyphosate (ISO); N-(phosphonomethyl)glycine

**EC Number: 213-997-4
CAS Number: 1071-83-6**

CLH-O-0000001412-86-149/F

Adopted

15 March 2017



15 March 2017

CLH-O-0000001412-86-149/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: glyphosate (ISO); N-(phosphonomethyl)glycine

EC Number: 213-997-4

CAS Number: 1071-83-6

The proposal was submitted by **Germany** and received by RAC on **17 March 2016**.

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at <http://echa.europa.eu/harmonised-classification-and-labelling-consultation/> on **2 June 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **18 July 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC:

Christine Bjørge

Co-Rapporteur, appointed by RAC

Stine Husa

Members of the ad hoc working group appointed by RAC:

Radu Branisteanu
Anne-Lee Gustafson
Normunds Kadikis
Riitta Leinonen
Brendan Murray
Pietro Paris

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **15 March 2017** by **consensus**.



Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification Hazard Class and Category Code(s)	Labelling		Specific Conc. Limits, M-factors	Notes
						Hazard statement Code(s)	Pictogram, Signal Word Code(s)		
Current Annex VI entry	607-315-00-8	glyphosate (ISO); N-(phosphonomethyl)glycine	213-997-4	1071-83-6	Eye Dam. 1 Aquatic Chronic 2	H318 H411	GHS05 GHS09 Dgr	H318 H411	
Dossier submitters proposal	607-315-00-8	glyphosate (ISO); N-(phosphonomethyl)glycine	213-997-4	1071-83-6	Retain Eye Dam. 1 Aquatic Chronic 2 Add STOT RE 2	Retain H318 H411 Add H373	Retain GHS05 GHS09 Dgr Add GHS08	Retain H318 H411 Add H373	
RAC opinion	607-315-00-8	glyphosate (ISO); N-(phosphonomethyl)glycine	213-997-4	1071-83-6	Retain Eye Dam. 1 Aquatic Chronic 2	Retain H318 H411	Retain GHS05 GHS09	Retain H318 H411	
Resulting Annex VI entry if agreed by COM	607-315-00-8	glyphosate (ISO); N-(phosphonomethyl)glycine	213-997-4	1071-83-6	Eye Dam. 1 Aquatic Chronic 2	H318 H411	GHS05 GHS09 Dgr	H318 H411	



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GROUNDWORK FOR ADOPTION OF THE OPINION



HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) summarised more than 20 acute toxicity studies where exposure was via the oral route. The lowest dose resulting in mortality was 2500 mg/kg bw in both mice and rats, but the number of dead animals at this dose was low and many studies had demonstrated that most animals tolerated even much higher doses of ≥ 5000 mg/kg bw. Since the LD₅₀ values were consistently >2000 mg/kg bw, the DS concluded that classification for acute oral toxicity was not warranted. The DS noted that clinical signs following oral exposure frequently included breathing difficulties, diarrhoea, reduced activity, ataxia, piloerection, convulsions and hunched posture.

In 21 acute toxicity studies summarised in which exposure in rats and rabbits was via the dermal route, the only death reported was one female rabbit receiving 5000 mg/kg bw. Isolated signs of toxicity comprised body weight loss, diarrhoea and slight local effects. Since the LD₅₀ values were all >2000 mg/kg bw the DS concluded that classification for acute dermal toxicity was not warranted.

In many of the 13 acute inhalation toxicity studies with glyphosate in rats summarised in the CLH report, a concentration ≥ 5 mg/L was tested. The DS therefore considered the information on effects of inhaled glyphosate at high concentrations to be sufficient despite this limit concentration not having been achieved in all experiments. Mortality was confined to 2 studies (Rattray, 1996, and Nagy, 2011), but the LC₅₀ value in these studies was ≥ 5 mg/L and hence the DS concluded that classification for acute inhalation toxicity was not warranted. Clinical signs included irritation of the upper respiratory tract, hyperactivity, increased or decreased respiratory rate, piloerection, loss of hair, wet fur, slight body weight reduction, slight tremor and slight ataxia, but the DS noted that these findings were not observed consistently in the studies.

Comments received during public consultation

A single reference to a published study addressing this endpoint (included in the renewal assessment report (RAR)) was submitted during public consultation.

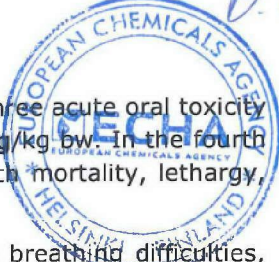
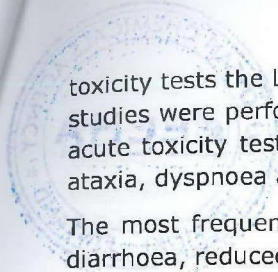

Assessment and comparison with the classification criteria

Animal data

The DS has included several acute toxicity studies, mostly in rats following oral, dermal and inhalation exposure. In addition, studies in mice following oral exposure, and in rabbits following dermal exposure were also included.

Oral exposure

For the assessment of acute toxicity following oral exposure to glyphosate, 24 studies in rats (and 4 in mice) were included by the DS (Table 10, CLH report). Ten of the acute toxicity tests were performed with only one concentration (limit test or fixed dose test) with LD₅₀ values > 2000 mg/kg bw and 10 with an LD₅₀ value of > 5000 mg/kg bw. In the remainder of the acute



toxicity tests the LD₅₀ values ranged from >5000 to > 8000 mg/kg bw. Three acute oral toxicity studies were performed in mice as limit tests with LD₅₀ values > 2000 mg/kg bw. In the fourth acute toxicity test in mice an LD₅₀ value > 7500 mg/kg bw was set with mortality, lethargy, ataxia, dyspnoea and weight loss observed at ≥ 2500 mg/kg bw.

The most frequent toxic signs reported in the acute toxicity tests were breathing difficulties, diarrhoea, reduced activity, ataxia, piloerection, convulsions and hunched posture. Mortality was reported in one study in rats with mortality in 1/10, 1/10, 3/1, 7/10 and 10/10 animals at 2500, 3500, 5000, 7000 and 9000 mg/kg respectively. In mice mortality was also reported in one study from ≥ 2500 mg/kg bw.

RAC concludes that following oral exposure to glyphosate, LD₅₀ values in rats and mice were consistently above 2000 mg/kg bw which, according to the CLP regulation, is the upper threshold for classification for acute toxicity following oral exposure. Therefore, **no classification for acute toxicity via the oral route is justified.**

Dermal exposure

For the assessment of acute toxicity following dermal exposure to glyphosate, 20 studies in rats and one in rabbits were included by the DS (Table 11, CLH report). Eighteen of the studies in rats were performed with one high dose of glyphosate (limit test) with LD₅₀ values > 2000, > 5000 or > 5050 mg/kg bw. In two studies with several doses of glyphosate the LD₅₀ values were > 5000 or 8000 mg/kg bw. No mortality was reported in the studies. In rabbits the LD value was > 5000 mg/kg bw, with mortality at day 14 in one female rabbit at 5000 mg/kg bw which was not related to glyphosate exposure.

The most frequent toxic signs reported in the acute toxicity tests were body weight loss, diarrhoea and slight local effects.

RAC concludes that following dermal exposure to glyphosate, LD₅₀ values in rats and rabbits were consistently above 2000 mg/kg bw which, according to the CLP regulation is upper threshold for classification for acute toxicity following dermal exposure. Therefore, **no classification for acute toxicity via the dermal route is justified.**

Inhalation exposure

For the assessment of acute toxicity following inhalation exposure to glyphosate, 13 studies in rats were included by the DS (Table 12, CLH report). In eight of the studies only one concentration at approximately 5.0 mg glyphosate /L was tested and all LC₅₀ values were ≥ 5.0 mg/L. Of the remaining studies, two studies were performed with a concentration of glyphosate at approximately 2.0 mg/L with LC₅₀ values > 2.0 mg/L and one study with an LC₅₀ value of > 3.25 mg/L. Two studies had two concentrations of glyphosate with LC₅₀ values > 2.88 mg/L and > 4.43 mg/L, respectively, the highest concentration tested.

The most frequent toxicological signs reported in the acute toxicity tests were irritation of the upper respiratory tract, hyperactivity, increased or decreased respiratory rate, piloerection, loss of hair, wet fur, slight body weight reduction, slight tremor and slight ataxia. The clinical signs were not reported consistently among the studies. Mortality was reported in two studies; in the first study, 2/5 males and 2/5 females died at 4.43 mg/L; in the second study, only 1/5 females died at 5.04 mg/L. The incidence of deaths in the two studies did not result in LC₅₀ values below 5.0 mg/L. Both studies used glyphosate from the same source.

RAC concludes that following inhalation exposure to glyphosate no LC₅₀ values in rats were reported to be below 5.0 mg/L which, according to the CLP regulation is the upper threshold for classification for acute toxicity (dust and mists) following inhalation exposure. Therefore, **no classification for oral toxicity via the inhalation route is justified.**

Human data

In the CLH report, no studies or case reports were found where humans were exposed to glyphosate itself at acute doses. However, a number of poisoning incidents have been reported following accidental or intentional intake of formulated glyphosate-based herbicides, mostly via the oral route but also some by inhalation. The doses in these poisoning incidents were not reported, however the DS estimated the intake of glyphosate from a few intoxication cases via the oral route where fatalities were observed, to be above 2000 mg/kg bw.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Based on a number of acute toxicity studies in rats and mice, in which non-lethal effects were confined to very high doses and were non-specific, the DS concluded that classification for STOT SE (categories 1 or 2) was not appropriate. In support of this argument, no evidence of neurotoxicity was observed in an acute neurotoxicity study in rats at doses up to 2000 mg/kg bw.

The DS also concluded that no classification for respiratory irritation was warranted (STOT SE (category 3)), since there was no evidence for respiratory tract irritation by the active substance in humans, but acknowledged that "such an exposure will seldom occur". The DS suggested that reported cases of possible respiratory irritation were from formulations containing polyoxyethylenealkylamine (POEA) surfactants. There was, however, no data to confirm if this was indeed the case.

The DS further noted that there was no evidence of narcotic effects observed in any of the evaluated studies.

Comments received during public consultation

No comments addressing this endpoint were submitted during public consultation.

Assessment and comparison with the classification criteria

Several acute toxicity studies in rats and mice were briefly described by the DS to illustrate transient, non-lethal and unspecific effects (associated with high doses of glyphosate) that were not sufficient for classification with STOT SE 1 or 2. Supporting evidence was also found in an acute neurotoxicity study in rats where no neurotoxicity was reported at dose levels of 500, 1000 and 2000 mg/kg bw. Furthermore, no clinical signs were reported after the first exposure from many repeated dose toxicity studies where lower doses were applied.

As regards classification with STOT SE 3 (narcotic effects), no narcotic effects were reported in any of the toxicity studies.

Further consideration was given to a classification with STOT SE 3 for respiratory irritation. Clinical signs were reported in a variety of acute inhalation studies performed on rats. Vague and general effects on breathing were described as clinical signs in 8 out of 13 inhalation toxicity studies according to the CLH report and the 2013 RAR. These effects were not consistent. The studies were all performed with glyphosate acid and were all guideline (and GLP) compliant. Two studies (Rattray, 1996, and Nagy, 2011) had mortalities and clinical signs were more pronounced.

Pathology findings (dark lungs) were reported in one study (Rattray, 1996) but not in the other. The remaining studies except for Tornai (1994) (which reported congestion, haemorrhage and oedema in the lungs), showed no pathological findings (10 studies).

There was no evidence of respiratory tract irritation in humans following exposure to glyphosate. In one study described by the DS (Burger *et al.*, 2009), one case of respiratory tract irritation was considered to be due to exposure to a formulated mixture and not solely the active substance glyphosate. The authors speculated that the effect was due to polyethoxylated alkylamine (PEEA) nonionic surfactants. In any case, this particular study did not provide any significant information to compare with the classification criteria.

In summary, there was no human data to support classification for respiratory tract irritation. There were no objective measurements of clear respiratory tract irritation. A variety of clinical signs were observed across a number of acute studies (slight dyspnoea, decreased respiratory rate, increased respiratory rate, breathing effects, irregular breathing, rales, laboured respiration, gasping respiration), but they were not always consistent and did not always occur together but in isolated studies. There is a general lack of pathology examinations in the studies (lung pathology was recorded in only 2 out of 13 studies) and it is difficult to rule out the possibility that isolated idiosyncratic reactions or responses triggered in hypersensitive test subjects were being observed. All effects appear to have been transient in nature. It is therefore not possible to list a definable set of clinical signs that are characteristic amongst all the acute studies reported by the DS. In conclusion there is not sufficient evidence amongst these studies to meet the CLP criteria for classification.

RAC concludes that **classification for specific target organ toxicity – single exposure is not justified**, based on the results from the acute and the repeated dose toxicity studies when compared with the CLP criteria.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS reported that 9 out of 11 studies addressing skin irritating effects of glyphosate were "unequivocally negative", and the results from the remaining 2 studies (very slight erythema in one animal in each study that had cleared within 24 hours) did not suggest that classification was warranted. Therefore no classification was proposed for skin corrosion/irritation.

Comments received during public consultation

No comments addressing this endpoint were submitted during public consultation.

Assessment and comparison with the classification criteria

Eleven guideline-compliant studies with rabbits have been summarized by the DS (Table 13, CLH report). From these, 9 studies were negative. Two studies (Merkel, 2005, and Zelenak, 2011; both consistent with OECD TG 404) each showed very slight erythema with mean scores of 1 and 0.3 respectively in 1/3 animals when 0.5 g glyphosate was applied to intact skin. The erythema was reversed within 24 hours in one study and within 48 hours in the other. Classification is triggered where a mean value of $\geq 2.3 - \leq 4.0$ for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours is observed, and hence the results do not meet the criteria for classification for skin irritation category 2.

There is very limited information on skin irritation in humans. Where skin irritation has been reported, it is unclear whether it is related to glyphosate or co-formulants in glyphosate-containing herbicide formulations. Thus, there is insufficient human data to support classification.

In conclusion, RAC agrees with the DS that **no classification for skin corrosion/irritation** is warranted.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Glyphosate has an existing harmonised classification for Eye Damage (Category 1). The DS reported that eye irritation was observed in 9 out of 13 studies addressing effects of glyphosate on the eye, and one revealed corrosive properties, but the three remaining studies were negative for eye irritation. The DS noted, however, that in these studies, rinsing of the eyes was performed one hour after instillation, while according to OECD TG 405 the eyes should be rinsed after 24 hours. On the other hand, in many studies, there was no rinsing at all. The DS therefore assumed that the different outcomes could be explained by methodological differences.

The DS noted that the criteria for Eye Damage Category 1 were met in four studies, whereas the results from the other positive studies could instead support classifying glyphosate in category 2 (Eye Irritation).

The DS therefore concluded that since evidence of strong eye irritation was obtained in several (albeit not in all) studies, classification for Eye damage in Category 1 was warranted.

Comments received during public consultation

Four comments received during public consultation addressed this endpoint. Two member states and a government organisation agreed with the proposal to retain the current harmonised classification as Eye Dam. 1. A comment from Industry acknowledged that eye "irritation" is not unexpected with the glyphosate acid, but argued that it is used in formulations which contain glyphosate salts with a more neutral pH, study results from which "do not trigger classification for eye irritation". The DS responded that classification of the active substance for eye damage is needed, as concluded in the CLH proposal.

Assessment and comparison with the classification criteria

Glyphosate was classified in 1999 by the Technical Committee for Classification and Labelling (TC C&L) of the European Chemicals Bureau with Xi; R41 (Risk of serious damage to eyes). According to CLP, this classification corresponds to Eye Damage Category 1, H318 (Causes serious eye damage). Thirteen additional studies, not evaluated by the TC C&L, were presented by the DS. The studies assessed by the TC C&L group resulting in a classification with Xi; R41 were not included in the CLH report by the DS and were not assessed by RAC. A brief summary of the 13 studies not previously assessed are presented in the table below:

Eye irritation studies with technical glyphosate not previously considered for classification purposes

Study	Strain, number of Animals	Purity	Amount applied	Effects / Results
Kuhn (1996)	New Zealand White (NZW) rabbit, 6 males, 3 females	98.2%	0.1 mL (65 mg)	Severely irritant in unwashed eyes: corneal opacity, conjunctival redness, chemosis, not reversible within 21 days (2 females); moderate irritation in washed eyes (washed after 30s), reversible within 21 days. No scorings reported in the DAR so no clear conclusion can be drawn. However, according to the study report, this induced severe irritation.
Talvioja (2007); (study considered acceptable by DS)	NZW rabbit, 1 male, 2 females	95.1%	100 mg	Marked, early onset and transient ocular changes. Cornea opacity (mean scores; 0.67, 1.67, 2.0), conjunctival redness (mean scores; 2.0, 2.0, 2.67), chemosis (mean scores; 2.0, 2.0, 1.0), reversible within 10 days, no signs of corrosion or staining. Fulfils the criteria for category 2.
Leuschner (2009); (study considered supplementary by DS)	Himalayan rabbit, 3 males	96.4%	100 mg, rinsed 1h post application	Slight signs of ocular changes, reversible within 7 days. Not according to the current OECD TG 405 since rinsing of the eyes was done 1 hour after instillation. Results do not meet classification criteria.
Hideo (1995); (study considered acceptable by DS)	NZW rabbit, 12 females	97.56%	100 mg (pure)	6 females without eye irrigation. Cornea opacity (mean scores; 2.0, 2.67, 2.0, 2.0, 2.0 1.67, not reversible within 21 days (3/6 females)); iris lesions (mean scores; 1.0 (in 5 females), 0.67 (in one female), reversible within 10 days); conjunctival redness (mean scores 2.0 in females and reversible within 16 days); conjunctival chemosis (mean scores; 2.0, 1.67, 2.33, 2.33, 2.0, 1.67 in females and reversible within 7 days). 6 females with eye irrigation (30 sec. & 2 min. post application): reduced effects and faster recovery Fulfils the criteria for category 2.
Leuschner (2009); (study considered supplementary by DS)	Himalayan rabbit, 3 males	98.8%	100 mg rinsed 1h post application	Not according to the current OECD TG 405 since rinsing of the eyes was done 1 hour after instillation. Results do not meet classification criteria.
Leuschner (2010); (study considered supplementary by DS)	Himalayan rabbit, 3 males	97.3%	100 mg rinsed 1 h post application	Not according to the current OECD TG 405 since rinsing of the eyes was done 1 hour after instillation. Results do not meet classification criteria.
You (2009); (study considered acceptable by DS)	NZW rabbit, 2 males, 1 female	96.4%	0.1 mL (93.2 mg)	Cornea opacity, iris lesions, conjunctival redness & chemosis reversible within 9 days. The mean score of ocular reaction were 1.7 after 24 hours. Fulfils the criteria for category 2.
Merkel (2005); (study considered acceptable by DS)	NZW rabbit, 3 males	97.23%	0.1 mL (60 mg)	All animals: corneal opacity, iris lesions, conjunctival redness & chemosis, reversible within 10 days No scorings reported in the DAR. No clear conclusion can be drawn.
Canabrava Frossard de Faria (2008); (study considered acceptable by DS)	NZW rabbit, 1 male, 1 female	98.5%	100 mg	Only 2 animals due to severe effects: Corneal opacity, iritis, conjunctival hyperemia, edema and secretion. Effects in female not reversible within 21 days Fulfils the criteria for category 1.
Reagan and Laveglia (1988); (study considered acceptable by DS)	NZW rabbit 6 animals, likely 3/sex	97.76%	100 mg	One rabbit died: considered not treatment related. Corneal opacity (mean score 1-2,7), conjunctival redness, chemosis in 6/6 animals. Some effects not reversible within 21 days in 3/5 rabbits. Fulfils the criteria for category 1.

Johnson (1997); (study considered acceptable by DS)	NZW rabbit, 6 females	95.6%	100 mg	Corneal opacity (mean score 1.3), iritis (mean score 0.7), conjunctival redness (mean score 1.9) and chemosis (mean score 1.4). All effects reversible within 8 days. Fulfills the criteria for category 2.
Arcelin (2007); (study considered acceptable by DS)	NZW rabbit, 1 male, 2 females	96.1%	100 mg	Mild, early-onset and transient ocular changes (reversible within 7 days). Corneal opacity, mean score 0, iritis; mean score 0, conjunctiva redness; mean score 1.34, chemosis; mean score 0.44. Results do not meet classification criteria.
Tavaszi (2011); (study considered acceptable by DS)	NZW rabbit, 1 male	96.3%	Glyphosate technical 100 mg	Based on results in one animal, the study was terminated at 24 h; corneal opacity & erosion (3); conjunctiva: redness (3), chemosis (4), discharge (3), few black points; oedema of the eyelids; positive fluorescein staining at 24 h. Considered to fulfill category 1.

Three studies were negative for eye irritation. The other studies were unequivocally positive. The severity of eye irritation and reversibility of effects determines whether category 1 or category 2 classification is most appropriate.

The criteria for category 1 and 2 are described in Annex 1 of the CLP Regulation, Tables 3.3.1 and 3.3.2, respectively

Two studies by Canabrava Frossard de Faria (2008) and Reagan and Laveglia (1988) were considered as acceptable by the DS, and in these studies severe effects in the eyes of rabbits were reported and included corneal opacity, iritis, conjunctival hyperemia, chemosis and secretion that were not reversed after 21 days and the criteria for category 1 can be considered fulfilled. In the study by Tavaszi (2011) which investigated effects using one animal, the scores after 24 hours fulfilled the criteria for category 1 classification. Note, however, that the study was terminated after 24 hours, presumably due to the assumption that there was no expectation of reversibility for the observed severe effects.

Four other studies support classification in category 2. For the rest of the studies, no category can be assigned due to limited reporting of the data.

In summary, two studies fulfilled the CLP criteria for classification in category 1 and a third study was terminated before the usual observation time had ended, but the findings suggested that this category might be appropriate. Another group of studies fulfilled category 2 but with one of them the scoring was close to that for category 1. A third group of studies were negative. No clear correlation was observed between classification outcome and rinsing since studies with early rinsing (ranging from 30 seconds to 1 hour) and studies with rinsing at 24 hours or no reported rinsing met the criteria for either category 2 classification or no classification.

Humans experiencing contact with herbicides containing glyphosate have reported at least transient eye irritation to be a frequent symptom. It is however unclear if this is caused by the substance itself or if it can be caused or enhanced by co-formulants in the formulated product.

In conclusion, a number of studies of acceptable quality provided clear evidence that glyphosate met the criteria for classification as Eye Dam. 1. Overall, the results from the studies assessed for eye irritation/eye damage by RAC did not contradict the existing classification of Glyphosate in CLP Annex VI, and RAC agrees with the DS that a classification for **serious eye damage category 1 (H318; Causes serious eye damage)**, is justified and should be retained.



RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS noted that an appropriate animal model for respiratory sensitisation is not available and that there is no evidence of respiratory sensitisation in humans arising from exposure to formulations containing glyphosate.

Comments received during public consultation

Although this hazard class was not open for comment during public consultation, one comment from an individual referred to the role of surfactants in penetration of glyphosate through cellular barriers. Industry commented that 40 years of glyphosate use had not yielded evidence of respiratory sensitisation in humans.

Assessment and comparison with the classification criteria

Since no classification proposal was presented for this hazard class and no data was provided in the CLH report, it could not be assessed by RAC.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The 14 studies (Magnusson & Kligman Guinea Pig Maximisation Tests (GPMT) and Local Lymph Node Assays (LLNA)) addressing the skin sensitisation potential of glyphosate, which were summarised in the CLH report, were all negative. In addition, the DS noted that Buehler tests (not summarised in the CLH report) were also consistently negative. The DS therefore did not propose classification for skin sensitisation.

Comments received during public consultation

No comments were submitted during public consultation addressing this endpoint.

Assessment and comparison with the classification criteria

Two LLNA studies and 12 GPMT studies were included by the DS for the assessment of skin sensitisation (Table 15, CLH report). All studies were negative. In the GPMT studies the intradermal induction doses ranged from 0.01% to 10% and the vehicle was either arachis oil, propylene glycol, water, PEG-300, paraffin oil, white petrolatum, or isotonic saline. The challenge doses ranged from 15% to 75% glyphosate. In the LLNA studies the glyphosate acid dose levels used were 0, 10, 25 and 45 or 50 (%w/v). Hexylcinnamaldehyde was included as positive control and demonstrated sensitisation.

The DS also reported that Buhler tests performed with glyphosate were negative. However, information regarding these Buhler tests were not included in the CLH report because the results from the LLNA and GPMT studies were considered to be more rigorous than those from a Buhler test.

RAC concludes that based on the negative results from the GPMT and LLNA tests, **classification for skin sensitisation** is justified according to the CLP criteria.

RAC evaluation of specific target organ toxicity- repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS noted that although identification of toxic effects requiring classification and labelling for specific target organ toxicity – repeated exposure (STOT RE) is usually based on short-term (28 days, 90 days, in dogs also 1 year) or lifetime studies, other studies, such as those investigating reproductive or developmental toxicity, may also provide relevant information which may support a need for classification.

According to the CLH report, the pregnant rabbit was much more sensitive than other species to glyphosate with a much lower maternal NOAEL of 50 mg/kg bw/d and a LOAEL of 100 mg/kg bw/d, at which already mortality occurred in at least one study. The main findings were mortality, abortions, reductions in body weight (gain) and food consumption and gastro-intestinal clinical signs such as loose stool or diarrhoea.

In short-term and chronic studies in rats, mice, and dogs, toxic effects of glyphosate were confined to high doses. The DS noted that it seemed clear that no effects were anticipated in any species at doses below 300 mg/kg bw/d and that even at higher doses the effects were relatively minor but variable, differing between the studies or the same endpoint and in the same species, depending on strain, laboratory and (according to the DS) perhaps also test material (e.g. impurities). Treatment-related findings comprised lower body weight gain, slight alterations in clinical chemistry and haematological parameters as well as a lower urine pH and clinical signs that indicate gastrointestinal irritation or disturbance. More pronounced toxicity was only seen in a single dog study with capsule administration at the high dose (1000 mg/kg bw/day).

The DS concluded that based on the LOAEL of 100 mg/kg bw/day for maternal toxicity, including mortality, in pregnant rabbits, classification as STOT RE 2 was warranted.

Comments received during public consultation

Six comments received during PC (4 from MSCAs, 2 on behalf of an organisation) supported the proposal for classification as STOT RE 2. Two further comments on behalf of an organisation were in favour of no classification. Industry argued that the rabbit model cited as a basis for the proposed STOT RE classification is not relevant to humans in cases where nutritional integrity of orally dosed rabbits is compromised by gastrointestinal effects which result in loose stools, since this hinders coprophagy and this in turn results in poor nutrition, compromised health and even mortality. Furthermore, the maternal toxicity findings in rabbits were not considered by industry to be consistent with multiple studies conducted in mice, rats and dogs, which do not rely on coprophagy for a balanced diet.

The DS responded that due to the mortality observed, the pregnant rabbit was the most sensitive animal model and therefore argued for the proposal to classify glyphosate as STOT RE 2.

Assessment and comparison with the classification criteria

The DS included summaries of short-term studies, non-cancer effects in long-term studies and data on maternal toxicity from developmental toxicity studies in rabbits in their evaluation of

STOT RE. The developmental toxicity studies in rabbits are included since the classification proposed by the DS is based on mortality occurring in this animal species. As regards human information, no data were available according to the DS.

Short term toxicity studies

Glyphosate was tested in several oral short-term studies using rats, dogs and mice. In addition, some studies by the dermal route using rats and rabbits were also included in the CLH report.

Eight 90-day oral studies with rats demonstrated overall low toxicity of glyphosate (Table 16, CLH report). The study by Coles *et al.* (1996) reported a NOAEL of 79 mg/kg bw/d, with a corresponding LOAEL of 730 mg/kg bw/d. This was the lowest NOAEL observed amongst all the 90-day studies presented in the CLH report. Observations of soft stools and diarrhoea together with occasionally reduced body weight gain indicated that glyphosate caused some irritation to the gastrointestinal tract at high doses. Blood or haemoglobin in the urine and a decrease in urine pH was also observed. However, all these effects were observed at doses (starting from 569 mg/kg bw/d) well above the guidance values for classification for STOT RE (STOT RE 1: $C \leq 10$ mg/kg bw/d and STOT RE 2: $10 < C \leq 100$ mg/kg bw/d).

Four 90-day studies and four 1-year studies (Table 17, CLH report), showed that dogs have a similar sensitivity to glyphosate to that observed in the rat. However, in the 13-week dog study by Gaou (2007) animals showed severe signs of toxicity at 1000 mg/kg bw/d, including liquid/soft faeces, dehydration, thin appearance, vomiting and pallor, reduced feed consumption and effects on body weight. The maximum tolerable dose (MTD) was clearly exceeded in this study. In contrast, the 1-year dog study by Gobordhun (1991) showed only minor effects at the same dose level.

Studies in mice showed that the toxicity of glyphosate was similar to that reported for rats. The NOAEL was 1221 mg/kg bw/d in a 90-day study by Kuwahara (1995). The study by Perry *et al.*, 1991, reported no effects at the highest dose level of 4500 mg/kg bw/d. However, the study by Chan and Mahler (1992), reported a NOAEL of 500 mg/kg bw/d based on histological changes in the parotid gland seen at 1065 mg/kg bw/d and above. The parotid gland was not examined in the studies by Kuwahara (1995) and Perry *et al.*, (1991), however, no effects were noted for either the sublingual or submaxillary glands that were examined in these two studies.

In conclusion, the short-term studies showed effects at doses above the relevant guidance values for classification for STOT RE (STOT RE 1: $C \leq 10$ mg/kg bw/d and STOT RE 2: $10 < C \leq 100$ mg/kg bw/d).

Long-term studies (non-neoplastic effects)

A large number of long-term studies have been performed in rats and mice (Tables 25 and 30, CLH report). For neoplastic effects, see the carcinogenicity section. Occurrence of non-neoplastic effects in these studies can be relevant for classification for STOT RE. However, none of the long-term studies presented in the CLH report reported effects at dose levels relevant for classification with STOT RE (2-year study: STOT RE 1: $C \leq 2.5$ mg/kg bw/d and STOT RE 2: $2.5 < C \leq 25$ mg/kg bw/d). A 1-year study with rats (Milburn, 1996) observed effects on body weight, food consumption, food efficiency, alkaline phosphatase activity and focal basophilia of acinar cells of parotid salivary gland starting at 560 mg/kg bw/d in male rats. In at least three of the 2-year studies in rats and mice effects were seen starting at 300-400 mg/kg bw/d, whereas the LOEL was much higher in the remaining studies.



Maternal toxicity in developmental studies in rabbits

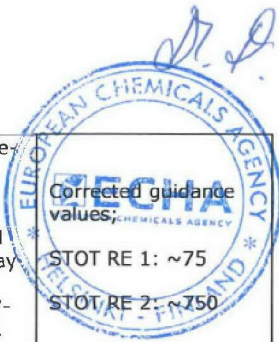
Findings from developmental toxicity studies can also be of relevance for classification for STOT RE. According to the CLP regulation (CLP Annex I section 3.9.2.5). Thus the use of the rabbit developmental studies for the assessment of STOT RE is considered justified by RAC

A wide range of studies are available; these include multi-generation studies in rats and developmental studies in rats and rabbits. The 2-generation studies with rats showed treatment related findings at very high doses, with reported NOAELs in the range of 200-700 mg/kg bw/d. The developmental studies showed NOAELs for maternal toxicity starting at 300 mg/bw/d, however for most studies, no effects on maternal toxicity were seen up to the limit dose for reproductive toxicity (1000 mg/kg bw/d; OECD TG 414).

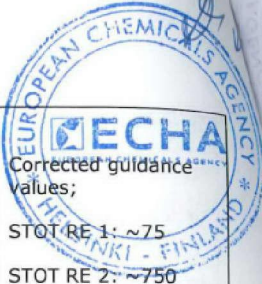
However, rabbits seem to be a much more sensitive species for effects arising from glyphosate exposure. Findings, including maternal deaths, are summarized in the table below.

Rabbit maternal mortality and toxicity from developmental studies with glyphosate.

Study, purity, strain, duration, dose levels, female rabbits per group	Premature deaths and cause of deaths*	Further maternal effects	Maternal NOAEL / LOAEL (mg/kg bw/d) Corrected Guidance values**
Tasker <i>et al.</i> , 1980; 98.7%, Dutch Belted rabbit, GD 6- 27, gavage, 0, 75, 175, 350 mg/kg bw/d 16 female rabbits per group (17 in high dose group) Study considered supplementary in RAR	<u>Found dead:</u> 1, 2, 10 at 75, 175 and 350 mg/kg bw/d. At 350 mg/kg bw/d 1 animal died prior to treatment, and was replaced. Out of these, 1, 1 and 3 deaths at 75, 175 and 350 mg/kg bw/d, respectively, were not regarded as being substance related (pneumonia, respiratory disease, enteritis or gastroenteritis). Cause of death could not be determined for remaining 8 animals. First death; Day 14 (350 mg/kg bw/d) Further deaths: Day 17, 18, 21 (350 mg/kg bw/d); 22, 25 (175 mg/kg bw/d); 26 (75 mg/kg bw/d) <u>Abortions:</u> 2 (GD 22), 1 (GD 27), 1 (GD 23) were sacrificed after abortion at 0, 175 and 350 mg/kg bw/d	Soft stool & diarrhoea (noted in all dose groups, but increased compared to control from 175 mg/kg bw/d). No treatment related effect on maternal bw and bw gain in female rabbits that survived to scheduled time.	75 / 175 Corrected guidance values; STOT RE 1: ~43 STOT RE 2: ~430
Bhide & Patil, 1989; 95%, NZW rabbit, GD 6-18, gavage, 0, 125, 250, 500 mg/kg bw/d, 15 female rabbits per group Study considered supplementary in RAR.	No mortalities observed.	Food consumption significantly reduced in high dose group. Body weight reduced in high dose group, no information regarding significance.	250 / 500 Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750
Brooker <i>et al.</i> , 1991; 98.6%,	<u>Found dead:</u>	Soft/liquid stool (2, 5, 13 animals at 50, 150 and	50 / 150



<p>NZW rabbit, GD 7-19, gavage, 0, 50, 150, 450 mg/kg bw/d, 16 – 20 female rabbits per group Study considered acceptable in RAR.</p>	<p>1 premature death at 450 mg/kg bw/d on day 20. Mortality occurred after cessation of treatment and signs of abortion GD 19, signs of gastrointestinal disturbance, severe reduction in food consumption and bodyweight loss.</p> <p>Two other deaths were unrelated to the treatment (broken hindleg at 450 mg/kg bw/d and congenital abnormality in control group)</p> <p><u>Abortions:</u> 1 at 50mg/kg bw/day (whole litter). 1 at 150 mg/kg bw/day (aborted 1 of 9 fetuses, remaining litter values are included in assessment).</p>	<p>450 mg/kg bw/d) (dose-related increase).</p> <p>Reduced food consumption compared to the control (12 % day 11-19 at 150 mg/kg bw/d and 6-17% day 7-19 at 450 mg/kg bw/d.</p> <p>A slight reduction in bw gain from GD 11 to termination at 150 and 450 mg/kg bw/d.</p>	<p>Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750</p>
<p>Suresh <i>et al.</i>, 1993; 96.8%, NZW rabbit, GD 6-18, gavage, 0, 20, 100, 500 mg/kg bw/d, 15 – 17 female rabbits per group in treated groups, 26 in control Study considered supplementary in RAR.</p>	<p><u>Found dead:</u> Premature deaths; 2 (control) died due to misgavage. 4 (100 mg/kg bw/d), 8 (500 mg/kg bw/d),) died from treatment, however several of these animals were shown to have pathological changes in the lungs.</p> <p>First death; Day 7 (2x 100 mg/kg bw/d; 1x 500 mg/kg bw/d)</p> <p>Further deaths: Day 9, 18 (100 mg/kg bw/d) 11, 14, 15, 18, 19 (500 mg/kg bw/d)</p> <p><u>Abortions:</u> No information regarding abortions.</p>	<p>At 500 mg/kg bw/d: Soft/liquid stool (stat. sign). Significantly reduced food consumption (31%, day 6-19). Significantly reduced maternal body weight and body weight gain. Toxicity symptoms involving rales, dyspnoea and weakness.</p>	<p>20 / 100 Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750</p>
<p>Hojo, 1995; 97.56%, Japanese White rabbit (Kbl:JW), GD 6-18, gavage, 0, 10, 100, 300 mg/kg bw/d, 18 female rabbits per group Study considered acceptable in RAR.</p>	<p><u>Found dead:</u> 1 dead at 300 mg/kg bw/d (no clinical signs), day 20</p> <p><u>Abortions:</u> Abortions; 2 at 10mg/kg bw/d (day 20, premature delivery day 27), 2 at 300 mg/kg bw/d (day 26, premature delivery day 27).</p>	<p>4 animals showed loose stool in the high dose group. Loose stool were also seen in two control animals and in one animal in the low dose group. No significant effect on food consumption and body weight.</p>	<p>100 / 300 Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750</p>
<p>Coles and Doleman, 1996, 95.3%, NZW rabbit, GD 7-19, gavage, 0, 50, 200, 400 mg/kg bw/d, 18 female rabbits per group Study considered acceptable in RAR.</p>	<p><u>Found dead:</u> 2 at 400 mg/kg bw/d (day 19 and 20). One found dead, one killed in extremis. 1 in control found dead after dosing 1 at 200 mg/kg bw/d found dead day 16 (mal-dosing)</p> <p><u>Abortions:</u> The animal killed in extremis day 20 showed signs of abortion.</p>	<p>Scours. At 400 mg/kg bw/d stat. sign. ↓ in food consumption from GD 10-19 and ↓ bw gain from day 9-29 stat. sign. from day 13. Vaginal bleeding and blood on tray were noted for 1 animal at 200 mg/kg bw/d.</p>	<p>50 / 200 Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750</p>
<p>Moxon, 1996; 95.6%,</p>	<p>Abortions; 1 in control (day 30), 2 at 100 mg/kg bw/d (day 19 and 25), 1 at</p>	<p>Diarrhoea, ↓ food consumption accompanied</p>	<p>100 / 175</p>

NZW rabbit, GD 8-20, gavage, 0, 100, 175, 300 mg/kg bw/d, 20 female rabbits per group Study considered acceptable in RAR.	175 mg/kg bw/d (day 22), 2 at 300 mg/kg bw/d (day 23 and 24). 1 at 175 mg/kg/bw/d killed for humane reasons (day 23) following bw loss and reduced food consumption.	by a stat. sign. ↓ bw gain in high dose group from GD 17-26	 Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750
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* There is a lack of consistency between the studies in how an animal that aborted is "labelled" i.e. it was either described as "killed in extremis" or "killed due to abortion" and sometimes an animal that was "found dead" had shown signs of abortion. However, in many cases all these "labels" can at least partly be viewed as just representing different expression of the same toxicity.

** CLP 3.9.2.9.8: "Guidance values are intended only for guidance purposes i.e. to be used in a weight of evidence analysis. They are not intended as strict demarcation values". In rabbits the perturbed digestion alters the absorption of glyphosate thus influencing the actual dose absorbed from the GI tract.

GD = gestation day

Five out of the 7 studies presented in the table above showed premature maternal deaths. These maternal deaths cannot be considered to reflect an acutely toxic effect since they occurred after several days of treatment. In 3 studies (Tasker *et al.*, 1980; Suresh *et al.*, 1993; Coles and Doleman, 1996) reporting premature death, the cause of death for some animals was suggested to be due to misgavage. The presence of premature deaths was observed in female rabbits along with decreased food consumption and reduced bw gain in 4 of the 5 studies. However, decreased food consumption and reduced bw gain were also reported in female rabbits without premature death at similar doses of glyphosate to those administered in the studies with premature death. Therefore, the premature death reported is not considered to be only related to decreased food consumption and reduced bw gain. Soft/liquid stool and diarrhoea was also a consistent feature reported in most of the rabbit developmental toxicity studies indicating a local irritating effect of glyphosate in the gastrointestinal tract. It was reported in female rabbits from studies with both a high level of premature deaths and in studies with none or low levels of maternal premature deaths. Therefore, a clear association between the premature maternal deaths and soft/liquid stool and diarrhoea cannot be established. Since in some of the studies the cause of some of the premature deaths was not clear (i.e. due to problems with the dosing technique or due to infections), and soft/liquid stool were also in some cases reported for controls, no clear association between premature death and these effects could be established. These clinical signs were also reported in some of the 2-generation and developmental toxicity studies in rats following repeated exposure to glyphosate without leading to death of the animals.

Caecotrophes are the material resulting from the fermentation of food in the rabbit caecum. They are nutrient-rich and are passed out of the body, like faeces, but are reingested by the animal so the nutrients can be absorbed. Several of these studies reported that the rabbits showed soft stools and/or diarrhoea. Maternal toxicity can be related to soft stools and diarrhoea because these effects may prevent the rabbits from eating their caecotrophs, often an essential, specialised digestive strategy for the recycling of caecal contents and the extraction of nutrients. However, studies of rabbits completely deprived of caecotrophs demonstrate that while caecotrophy is very important for normal growth, it is not always essential for survival (Robinson *et al.*, 1985; Phiny *et al.*, 2006). In the studies detailed above there is no information that the animals were not able to eat their caecotrophes. If the animals are ingesting their caecotrophes, one could anticipate that female rabbits will be exposed to unmetabolised glyphosate repeatedly since glyphosate, is excreted unchanged via faeces (<http://www.nutrecocanada.com/docs/shur->

gain---specialty/caecotrophy-in-rabbits.pdf). Therefore, the recirculation of digestive material containing glyphosate will have an influence on the actual dose absorbed from the GI tract.

According to the CLP criteria, all available evidence, and effects relevant to human health, shall be taken into consideration in the classification process. This can include morbidity or death resulting from repeated or long-term exposure. The guidance values for classification in category 1 for a 90-day oral exposure study in rats is less than 10 mg/kg bw/d, and for a 28-day study less than 30 mg/kg bw/d. The guidance value for classification in category 2 is less than 100 mg/kg bw/d for a 90-day oral exposure study, and less than 300 mg/kg bw/d for a 28-day study. However, according to CLP (Annex I, 3.9.2.9.8), "*Guidance values are intended only for guidance purposes i.e to be used in a weight of evidence analysis. They are not intended as strict demarcation values*". There are no guidance values specified for oral exposure of rabbits, but RAC considers that the guidance values for rats might be used as part of a weight of evidence also for other species, including rabbits.

For the evaluation of the rabbit developmental toxicity studies in the table above, the findings at particular doses have been compared with guidance values corrected for the duration of the exposure (according to Haber's rule). It can be seen from the table that all 5 studies showed premature deaths within the corrected guidance values for classification with STOT RE 2. However, it is important to take into account that guidance values are only for guidance purposes and that the perturbed digestion in the female rabbits may alter the absorption of glyphosate thus influencing the actual dose absorbed from the GI tract. Therefore, the use of Haber's rule to correct the guidance values in these studies includes uncertainties and the results should be used with caution.

In the Suresh *et al.* (1993) study, with a high level of premature deaths, two premature deaths were also reported in the control group and were confirmed to be due to mis- or mal-dosing. In the DAR (2015) some doubts were also raised relating to the four deaths reported at 100 mg/kg bw/d since there were no signs of toxicity at this dose level. In the other rabbit developmental toxicity studies no deaths was reported at similar dose levels, further contributing to doubts over the cause of the deaths reported at this dose level in the Suresh *et al.*, (1993) study. In addition, at gross necropsy various findings were noted in the lung and trachea in the mid- and high dose groups (100 and 300 mg/kg bw/d, respectively) in the female rabbits that died. In the high dose group microscopic examination showed that 5 out of 8 female rabbits had lung lesions (emphysema, collapsed, pneumonic lesions, consolidated and congested) and in the mid-dose group 1 out of 4 female rabbits that died had lung and trachea congestion and froth in the trachea suggesting that gavage errors could have contributed to some of the deaths reported at these dose levels.

In the study by Tasker *et al.* (1980), 3/10 mortalities at 350 mg/kg bw/d, 1 mortality at 175 mg/kg bw/d and 1 mortality at 75 mg/kg bw/d were reported to be due to pneumonia, respiratory disease, enteritis or gastroenteritis. Unfortunately, there was no necropsy report attached to the original study report and the cause of death for the remaining 7/10 animals in the high dose group and 1 animal at 175 mg/kg bw/d and 1 animal at 75 mg/kg bw/d were not reported with any degree of detail so it cannot be ascertained if it was substance related or not. Premature deaths were also reported in the studies by Hojo (1995); Coles and Doleman (1996) and Brooker *et al.* (1991), at doses from 300 to 450 mg/kg bw/d without reporting of mis-dosing, all with a lower incidence of mortality than reported in the studies by Tasker *et al.* (1980) and Suresh *et al.* (1993). There are some uncertainties remaining related to the cause of the premature maternal deaths in the studies by Suresh *et al.* (1993) and Tasker *et al.* (1980), since it is not clear if the deaths was attributable to exposure to glyphosate, related to mis-dosing or to infections (e.g pneumonia, respiratory disease). Altogether, RAC considers that the premature



maternal deaths reported in several rabbit developmental toxicity studies cannot be viewed as clear evidence of glyphosate toxicity following repeated exposure.

According to Annex I: 3.9.2.9.7 of CLP "Classification in Category 2 is applicable, when significant toxic effects observed in a 90-day repeated dose study...are seen to occur within..." a range of $(10 < C \leq 100)$ mg/kg bw/d via oral exposure in the rat. Applying Haber's rule for a study of shorter duration (28 days) allows for extrapolation of the guidance values to a range of $(30 < C \leq 300)$ mg/kg bw/d via the oral route. However, in this case the use of Haber's rule to correct the guidance values includes uncertainties and the results should be used with caution.

The DS described excessive maternal toxicity as a number of unscheduled, treatment-related deaths in 5 out of 7 rabbit developmental studies within a dose range of 100 to 500 mg/kg bw/d. On this basis the DS proposed classification as STOT RE 2. Certainly, large doses of glyphosate are associated with severe maternal toxicity and death in female rabbits. However, the overall weight of evidence for classification is unconvincing due to the following reasons:

1. Strictly, there are only 2 studies with deaths reported below the corrected guidance value, i.e. 4 female rabbits in the Suresh *et al.* (1993) study at 100 mg/kg bw/d and 8 female rabbits at 500 mg/kg bw/d, and 2 female rabbits in the Tasker *et al.* (1980) study at 175 mg/kg bw/d and 10 female rabbits at 350 mg/kg bw/d where several of the deaths in each study could be related to mal-gavage.
2. In the Suresh *et al.* (1993) study, pathological changes in the lungs were noted in one of the dead animals at the 100 mg/kg bw/d and were suggestive of gavage errors. The remaining 3 decedents in the 100 mg/kg bw/d dose-group had no abnormalities and there were no reported clinical signs at this dose level. Five out of 8 mortalities in the high dose group also displayed pathological changes suggestive of gavage errors. The remaining 3 decedents in the 500 mg/kg bw/d group had no abnormalities. Soft stool and diarrhoea was reported, however, a clear association with premature death cannot be established. There were also 2 mis-dosings in the concurrent controls. Overall the frequent reporting of pathological findings in the lung suggestive of gavage errors raises concern regarding the technical skills in dosing via oral gavage and consequently also on the inclusion of this study in the assessment of substance induced mortality.
3. In the Tasker *et al.* (1980) study 1, 1 and 3 premature deaths at 75, 175 and 350 mg/kg bw/d, respectively, out of 1, 2 and 10 premature deaths at these dose levels were reported to be due to pneumonia, respiratory disease, enteritis or gastroenteritis; the remaining death was unexplained.
4. Five of the studies included in the table "Rabbit maternal mortality and toxicity from developmental studies with glyphosate" with dosing over the range 50 to 450 mg/kg bw/d did not reveal signs of an increased mortality as observed in the study by Suresh *et al.* (1993) and Tasker *et al.* (1980).
5. The majority of deaths were associated with high doses of glyphosate and the majority of deaths were associated with 2 studies where the cause of death is unclear.
6. The physiology of digestion in the rabbit is in some ways unique. In rabbits, caecotrophy ensures that substances predominantly excreted unchanged in the faeces such as glyphosate are readily available for repeated oral uptake and constitute a potentially significant oral dose relative to other species including humans. This possible recycling of glyphosate and increased exposure in rabbits might explain the particular sensitivity of this species while at the same time casting

doubt over the relevance of oral dosing in rabbit studies for humans. However, there is a lack of information regarding whether the rabbits were able to eat their caecotrophes or not, and therefore it is not possible to have a clear picture of a possible recycling of glyphosate and consequently the actual dose absorbed from the GI tract, leading to uncertainties with using Haber's rule to correct the guidance value for a STOT RE classification in these studies.

7. Signs of digestive disturbances (soft/liquid stool and diarrhoea) were consistently reported in the rabbit studies (but also in rats at much higher doses). However, a clear association with premature maternal death cannot be established. The fact that the female rabbits appear to be uniquely sensitive compared to rodent dams further support the the caecotrophy hypothesis and weakens the argument for classification in this case.

Furthermore, an in-depth analysis of all the data from both the short-term and long-term toxicity studies only shows effects at high dose levels exceeding the extrapolated guidance values relevant for a classification with STOT RE.

Mortality in female rabbits has been used to justify the proposal for classification of glyphosate for STOT RE 2 by the DS. According to CLP, Annex I, section 3.9.2.7.3, morbidity or death resulting from repeated or long-term exposure can be taken into account for classification as STOT RE. However, CLP further states that "Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites".

Following exposure to glyphosate, mortality in rabbits is considered to either be related to mis-dosing, infections or diarrhea and the possible mechanism of caecotrophy and recycling of glyphosate. No mortalities were recorded in the rat studies. In addition, bioaccumulation and over-whelming of detoxification mechanisms by repeated exposure as a mechanism of toxicity is not likely for glyphosate.

On the basis of a weight of evidence approach and with due consideration of all data from the short-term, long-term, reproductive and rabbit developmental studies, RAC concludes that **STOT RE classification is not justified** for glyphosate.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS summarised numerous *in vitro* studies with glyphosate, including standard bacterial assays and mammalian cell gene mutation tests, which gave consistently negative results. The DS also noted that the majority of *in vitro* chromosomal aberration tests and micronucleus tests were negative, and in particular, all of the studies performed under GLP conditions resulted in negative findings. No evidence of chromosome aberrations were obtained in 11 guideline-compliant *in vivo* micronucleus assays or chromosome aberration studies in which the bone marrow of either mice or rats was examined after oral or intraperitoneal application.

The DS also noted that in published studies with methodological limitations, the results were contradictory and that 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" according to the DS.

Evidence of exposure to glyphosate was based on the affinity of glyphosate to bone tissue as shown in the toxicokinetic studies, by the occasional observation of bone marrow toxicity in the tests themselves and by the occurrence of hypoplasia in bone marrow in a long-term study in rats (at a very high dose).

Positive results were observed for induction of sister chromatid exchange (SCE) and DNA strand breaks (comet assay) but a negative result in a study investigating induction of DNA repair (unscheduled DNA synthesis; UDS).

Based on a weight of evidence determination, the DS proposed no classification for germ cell mutagenicity.

Comments received during public consultation

One MSCA and one government authority supported classification as Muta. 2. The MSCA referred to positive findings in liver tissue of DNA damage in Comet assays and in studies of DNA strand breaks and DNA adducts in their argument. Three MSCA as well as industry agreed with the DS that classification for germ cell mutagenicity was not warranted.

One MSCA and one individual suggested that additional investigation be conducted, for example to clarify the mode of action (MoA) (including the role of oxidative stress and adduct formation) and investigation of genetic damage in workers.

Three comments submitted on behalf of an organisation considered that there was strong evidence of genotoxic properties of glyphosate as a mechanism for carcinogenicity.

Six individuals and one organisation supported classification without specifying a category.

Assessment and comparison with the classification criteria

Glyphosate has been tested in a wide range of genotoxicity assays. All genotoxicity studies included by the DS have been considered and both guideline and non-guideline studies form the basis of the current RAC mutagenicity evaluation. One additional genotoxicity study mentioned in the RAR, but not in the CLH report, was evaluated by RAC (Astiz, 2009) as it was also included in the International Agency for Research into Cancer (IARC) report (2015). Furthermore, a recent reproductive study mentioned in a comment from the PC (Dai *et al.*, 2016) is referred to by RAC as it included measurement of oxidative stress in the testis.

Glyphosate is not electrophilic, and is only metabolised to a limited degree as evidenced by the urinary excretion mainly of non-metabolised glyphosate. ADME studies show a wide tissue distribution of glyphosate following oral administration.

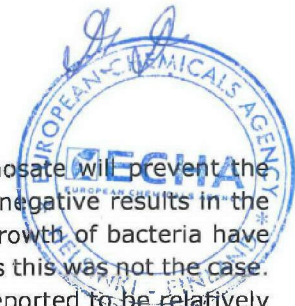
Germ cell mutagenicity tests

Glyphosate was tested in two germ cell mutagenicity tests (rodent dominant lethal tests), one in Wistar rats (Suresh, 1992) with single doses up to 5000 mg/kg bw and one in CD-1 mice (Wrenn *et al.* 1980) with doses up to 2000 mg/kg bw. Both were reported to be negative.

Mutagenicity and genotoxicity tests in bacteria and somatic cells

In vitro studies:

The ability of glyphosate to cause mutations in **bacteria** was tested in 16 Ames tests, the majority performed both with and without metabolic activation by a S9 pre-incubation step. All of these tests and one bacterial DNA repair assay (Rec-assay) were negative, indicating that glyphosate is not mutagenic or genotoxic in bacterial systems.



During the PC, a concern was raised that antimicrobial activity of glyphosate will prevent the growth of back-mutated *Salmonella*, thereby potentially producing false negative results in the Ames test. The DS responded that cytotoxicity or reduced background growth of bacteria have been reported in a few of the Ames tests at high doses, but in most studies this was not the case. Furthermore, in a study by Shehata *et al.* (2013), *S. typhimurium* was reported to be relatively resistant to the growth inhibitory effect of glyphosate (minimal inhibitory concentration of 5 mg/mL). The conclusion that glyphosate is negative in bacterial mutagenicity tests is thus considered valid.

In **mammalian cells** glyphosate was tested in a range of *in vitro* studies for mutagenicity, clastogenicity and DNA damage or repair.

Three **mammalian gene mutation** tests were reported; one CHO/HGPRT gene mutation assay (Li, 1983) and two mouse lymphoma tk locus assays (Jensen 1991; Clay 1996). Glyphosate was negative both with and without S9 metabolic activation at concentrations up to 5 mg/mL (current OECD TG 476/2016 requirement being 2 mg/mL) in the lymphoma assays and to 22.5 mg/mL in the Chinese hamster ovary (CHO) cells.


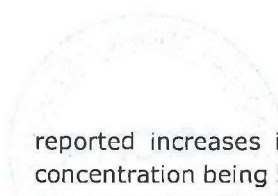
Two *in vitro* **micronucleus** tests were reported of which one was performed with human lymphocytes and was negative without S9 and positive in samples with S9 activation at the highest concentration tested (580 µg/mL; Mladinic, 2009). The second micronucleus test using a human buccal carcinoma cell line (TR146) exposed for a short period (20 minutes) to low glyphosate concentrations (10-20 µg/mL) was positive at the concentrations of 15 µg/mL and 20 µg/mL (Koller, 2012). At 20 µg/mL, increases in apoptosis and necrosis were reported, whereas the nuclear division index for cell integrity was reported to be unaltered by glyphosate exposure at these exposure levels. RAC notes that this cell line does not appear to be well characterised with respect to its performance in the *in vitro* micronucleus test.

Glyphosate did not induce **chromosomal aberrations** in five of the seven *in vitro* studies presented in the CLH report (Fox, 1998; Kyomu, 1995; Wright, 1996; Van de Waart, 1995; Mañas, 2009). The first three studies were reported as acceptable in the RAR, whereas the study by Van de Waart (1995) was used as a supplementary study as the top dose was not considered sufficiently high. In the study by Mañas *et al.* (2009) only 100 cells were scored per treatment reducing the power of the experiment. Positive results were reported in two chromosome aberration tests using bovine and human lymphocytes exposed to low concentrations of glyphosate (Lioi *et al.*, 1998a,b). These two studies were from the same laboratory and employed a non-standard exposure protocol. In the bovine study cytotoxicity appeared (55% reduction of mitotic index) even at the lowest concentration level. The test using human lymphocytes reported increases in chromosomal aberrations without any apparent reduction in mitotic index (Lioi, 1998b).

Three **SCE** tests were reported (Lioi 1998a,b; Bolognesi *et al.*, 1997) and all found evidence of increased levels of SCEs in glyphosate exposed lymphocytes.

One negative **UDS** assay using primary hepatocytes was presented in the CLH report (Rossberger, 1994). The UDS assay result suggests that glyphosate does not induce nucleotide excision repair. The assay is generally not sensitive towards detection of single-strand breaks and oxidative base lesions.

Five *in vitro* **Comet assays** were reported by the DS (Monroy *et al.*, 2005; Mañas *et al.*, 2009; Mladinic *et al.*, 2009b; Alvarez-Moya *et al.*, 2014; Koller *et al.*, 2012), and they were all positive. Monroy *et al.* (2005) observed a genotoxic effect in human fibroblasts and fibrosarcoma cells from concentrations at or above 4 mM. In the study by Mañas *et al.* (2009), DNA strand breaks were induced in Hep-2 cells of human epithelial origin at glyphosate concentrations between 507 and 1268 µg/mL (3-7.5 mM) with cytotoxicity at the highest dose level. Mladinic *et al.* (2009b)



reported increases in tail intensity or tail length from 3.50 µg/mL and above (the highest concentration being 580 µg/mL) in human lymphocytes both with and without S9. These findings were seen together with an increased rate of early apoptotic and necrotic cells, an indication of cytotoxicity. Alvarez-Moya *et al.* (2014) tested glyphosate in human lymphocytes and reported an increase in tail length at all tested concentrations from 0.118-118 µg/mL (0.7 up to 700 µM), but the differences in DNA strand breaks between the concentrations were small without a clear dose response relationship. Koller *et al.* (2012) studied the effects of glyphosate in a carcinoma cell line (TR146) of human buccal epithelial origin and reported an increase in tail intensity as compared to the controls at concentrations from 20 up to 2000 µg/mL, with an increase between 20 and 40 µg/mL and no apparent further change in response up to 2000 µg/mL.

In summary, the *in vitro* data are not entirely consistent, but indicate that glyphosate does not induce gene mutations. All Ames tests and mammalian gene mutation tests reported were negative. Five of the chromosomal aberrations tests were negative and two tests from the same laboratory, both following an alternative protocol and therefore given less weight in the assessment, were positive. The two micronucleus tests presented showed both positive and negative results, whereas the Comet assays indicate that glyphosate may induce DNA strand breaks or alkali labile sites in cultured cells.

The *in vitro* data have been corroborated by a range of *in vivo* genotoxicity and mutagenicity studies as described in the next section.

In vivo studies:

Non-human mammalian data

A considerable number of studies were available for the assessment of *in vivo* mutagenicity following exposure to glyphosate. These were bone marrow micronucleus and chromosome aberration tests in rats or mice after oral or intraperitoneal (i.p.) administration of glyphosate. Several toxicokinetics studies are presented in the RAR (B.6.1) and they indicated that glyphosate was widely distributed to body organs, including the bone marrow, although only low levels were measured.

Negative results were reported in 6 of the 7 **micronucleus tests** in bone marrow cells following **oral exposure** to glyphosate. The maximum doses for these studies were 2000 mg/kg bw or 5000 mg/kg bw given as single or double exposures, and all were performed according to OECD TG 474 and GLP. One micronucleus test, performed by Suresh (1993), demonstrated a statistically significant increase in the incidence of micronuclei in females at the high dose of 5000 mg/kg bw administered on two consecutive days (% micronucleated polychromatic erythrocytes (MN-PCE): control 0.51; high dose 1.05), but not in males (%MN-PCE: control 0.69; high dose 0.89). RAC notes that the control MN-PCE frequencies reported are higher than expected for this test. No increase in the percentage of micronuclei were observed at the low or middle doses in the same study. No historical control data for this study is mentioned in the CLH report. No effects on the PCE/ normochromatic erythrocytes (NCE) ratio were reported in any of the oral micronucleus studies.

In addition to the oral studies, seven mouse micronucleus tests in bone marrow cells were included by the DS following i.p. administration of glyphosate (from 15.6 to 563 mg/kg bw). Four of the studies showed no statistically significant increases in micronuclei (two of these performed according to OECD TG 474 and GLP). One study (Durward, 2006) was considered to be negative, although reporting a statistically significant increase in %MN-PCEs at the high dose of 600 mg/kg bw (single dose). The level of MN-PCEs at the high dose (mean %MN-PCE in control 0.06 and 0.19 in high dose) was within the historical control range, as indicated in Table 23 in the CLH report. Two micronucleus tests showed positive results. In the first positive study (Mañas *et al.*, 2009) Balb-C mice (5 per dose, sex unclear) were used. A statistically significant increase in

micronucleated erythrocytes (% MN cells in controls 0.38 and at high dose 1.3) was reported at 24 hours after the animals had received two i.p. doses of 200 mg/kg bw glyphosate, administered 24 h apart. The two lower doses (2x50 or 2x100 mg/kg bw) were negative in this study. The study was reported by the DS to have some deviations from the OECD TG 474, the most problematic being that 1000 (instead of 2000) erythrocytes per animal were scored, and "erythrocytes" instead of immature or "polychromatic erythrocytes" (PCE) were scored for micronuclei. RAC notes that it is unclear whether the authors have counted mature or immature erythrocytes as they did not specify this in the article. RAC also notes that counting as few as 1000 PCE (assuming PCE were counted) would give results which are less reliable. For these reasons, the result from this study should be interpreted with care. In the second positive study (Bolognesi *et al.*, 1997) an increase (0.075% in control; 0.14% at 6h and 0.24% at 24h) in micronuclei in mouse bone marrow cells following two i.p. doses of 150 mg/kg bw on two consecutive days was reported. The study is limited in its methodological description. However, it reports 4 animals (instead of five) in each of the glyphosate exposure groups, but counting of more cells (3000 vs 2000 NPCs per animal). The publication gives no reference to historical control data.

Two **chromosomal aberration** tests are reported in the CLH report, both of which were negative: In the study by Li and Long (1988) no chromosomal aberrations were induced in rat bone marrow following i.p. exposure to 1000 mg/kg bw glyphosate with sampling 6, 12 and 24 h after administration. In the second study in mouse (Suresh *et al.*, 1994), oral exposure to glyphosate at doses up to 2 x 5000 mg/kg bw did not induce an increase in chromosomal aberrations.

Human data

The CLH report refers to the EU-RAR, Section B.6.4.8.7 (page 417) for a description of genotoxicity studies in human populations with occupational exposure to glyphosate-based herbicides or exposure of bystanders/area residents. Some of the studies presented in the RAR suggest a higher level of MN and DNA strand breaks in association with glyphosate based herbicide exposure (Table B.6.4-30 and 4 additional studies mentioned in the RAR). The majority of the studies showed no such association or the reported glyphosate based herbicide usage by the studied population was too low to be associated with observed population effects. In some of the studies, high incidence not only of GHB use, but also of other pesticides was reported.

RAC finds that the interpretation of the human studies for the assessment of the genotoxicity of glyphosate is challenging due to the limited data available and confounding factors such as exposure also to other pesticides as well as uncertain exposure estimates. In addition, there is an issue with potential toxicity related to glyphosate based herbicide co-formulants.

Some evidence for genotoxicity was suggested in two published studies (described below) which investigated populations believed to be exposed to glyphosate based formulations.

Paz-y-Miño and co-workers (2007) examined the consequences of aerial spraying with a glyphosate based herbicide added to a surfactant solution in the northern part of Ecuador. A total of 24 exposed and 21 unexposed control individuals were investigated using the Comet assay 2 weeks to 3 months following intensive aerial spraying. The results showed a higher degree of DNA strand breaks in the exposed group. However, individuals among the exposed group manifested clinical symptoms of toxicity after several exposures to aerial spraying which may by itself have an effect on generation of DNA single strand breaks.

Bolognesi and co-workers (2009) reported on a binucleated MN biomonitoring study in subjects from five Colombian regions, characterized by different exposures to glyphosate and other pesticides. Blood samples were taken prior to spraying, 5 days and 4 months after spraying and a significant increase in the frequency of MN between first and second sampling was observed in three of the regions. In the post-spray sample, those who reported direct contact with the

weedkiller spray showed a higher frequency of MN compared to those without glyphosate exposure. The increase in frequency of MN observed immediately after the glyphosate spraying was not consistent with the rates of application used in the regions and there was no association between self-reported direct contact with eradication sprays and frequency of MN. Mañas *et al.* concluded that the data suggested that genotoxic damage associated with the glyphosate spraying as evidenced by the MN test was small.

Mammalian in vivo indicator tests

Comet assay/alkaline elution assay

Two *in vivo* assays have been reported that measured the formation of DNA strand breaks and alkali labile sites in blood cells, liver and kidney. An OECD test guideline (OECD TG 489) for the *in vivo* rodent Comet assay has recently been adopted and the assay has been validated by JaCVAM (Uno, 2015).

In the study by Bolognesi *et al.* (1997), DNA strand breaks were measured by the alkaline elution assay in mouse liver and kidney cells 4 h and 24 h following single i.p. administration of glyphosate (300 mg/kg bw). A transient induction of single strand breaks was detected at the 4 h time point.

In a study by Mañas *et al.* (2013), induction of DNA strand breaks was examined in mouse peripheral blood cells and liver cells as measured by the Comet assay following exposure to doses of approximately 40 and 400 mg/kg bw/d glyphosate via drinking water for 14 days. In this study an approximate doubling of the tail intensity measure was reported, with a dose-response relationship for liver cells. The methodological description in this publication is limited. These two studies suggest that glyphosate may induce increases in DNA strand breaks that are rapidly repaired following a single exposure. That glyphosate may induce increases in DNA strand breaks is supported by the *in vitro* comet assays, but the data also appear to show that the increase in strand breaks reach a plateau with no further increase with increasing dose. The biological significance of a slight increase in DNA strand breaks as demonstrated in the drinking water study (Mañas *et al.*, 2013) is uncertain.

Mechanistic studies - oxidative stress:

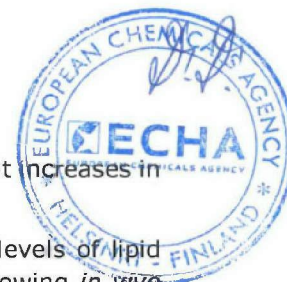
Measurements of DNA adduct levels and markers of oxidative stress may provide information on the potential genotoxic mode of action.

Bolognesi *et al.* (1997) measured formation of the oxidative DNA lesion 8-hydroxy-2'-deoxyguanosine (8-OHdG) in liver and kidney from mice 8 h and 24 h following a single i.p. exposure to glyphosate (300 mg/kg bw). A statistically significant increase in 8-OHdG was reported in liver at 24 h, but not after 8 h and not in the kidney.

No increase in DNA adduct formation was detected by the ³²P-postlabelling method following i.p. exposure to glyphosate isopropyl ammonium salt to mice at a single dose of 130 or 270 mg/kg bw (Peluso *et al.*, 1998).

Oxidative stress is characterized by an imbalance between generation of reactive oxygen species and anti-oxidant defense mechanisms, and can be measured as an increase in markers of oxidative stress such as malondialdehyde (MDA) e.g. by the thiobarbituric acid reactive substances (TBARS) assay.

In a study by Mladinic *et al.* (2009) exposing isolated human whole blood samples to glyphosate *in vitro*, several markers of oxidative stress were examined. In this study an increase in plasma TBARS levels was demonstrated at the highest concentration of 580 µg/mL glyphosate. A modified version of the comet assay was used with addition of the human 8-oxoguanine DNA



glycosylase (hOgg1) that recognises the oxidised DNA lesion 8-OHdG. No consistent increases in Ogg1-sensitive DNA lesions was revealed over the concentration range tested.

A few studies (Mañas *et al.*, 2009 and 2013; Dai *et al.*, 2016) have measured levels of lipid peroxidation byproducts (MDA/TBARS) as putative makers of oxidative stress following *in vivo* exposures of mice or rats to glyphosate. Significant changes in MDA or TBARS were not reported in mouse tissues to single or repeated administrations of glyphosate, although some differences in activities of antioxidant enzymes were reported (Mañas *et al.*, 2009 and 2013). In a rat study (Dai *et al.*, 2016) with doses up to 500 mg/kg bw/day for five weeks, no significant increases in testicular MDA levels or changes in anti-oxidant enzyme levels were reported. In addition, the IARC report and the RAR both refer to a study in rats by Astiz *et al.* (2009). This study measured effects on oxidative stress markers and oxidative defense systems in several tissues following repeated i.p. (10 mg/kg bw) glyphosate exposures three times a week for five weeks. TBARS concentrations in several tissues were increased (~doubled) in glyphosate exposed animals compared to the control animals, whereas plasma protein carbonyl levels were unaffected. In the RAR, this study is given Klimisch code 3 due to deficiencies in reporting, low number of animals per group (4 rats/group), and i.p. route of administration. RAC notes that only the unexposed control data and not the vehicle control data are presented and that the statistical evaluation seems to compare responses with the unexposed control data. The authors stated that they did not find any differences between data from the unexposed control group and the vehicle control group, but this is not shown.

In conclusion, the *in vitro* and *in vivo* data suggest that glyphosate may induce oxidative stress. However, increased levels of oxidative stress were not reliably demonstrated in the repeated dose studies where this was examined.

A number of organisations, international (WHO/JMPR), EU (EFSA) and national (for example US EPA, Australian APVMA) have assessed or are in the process of assessing the carcinogenic potential of glyphosate. So far, only IARC has concluded that glyphosate is genotoxic. Therefore a detailed comparison of the genotoxicity evaluation conducted by IARC and the DS is provided below.


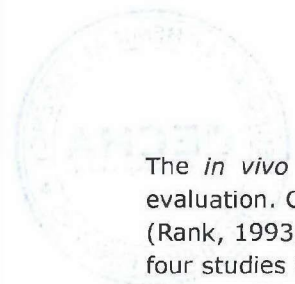
Comparison with the IARC evaluation

The IARC report is based on publicly available studies and does not consider data from unpublished reports, whereas the CLH report and the RAC opinion are based on both unpublished reports and publicly available studies resulting in a much broader data set for *in vivo* mammalian genotoxicity studies. In contrast to the RAC opinion, the IARC report includes studies in non-mammalian animal species.

IARC in their recent monograph 112 concluded:

"There is strong evidence that glyphosate causes genotoxicity. The evidence base includes studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms. In-vivo studies in mammals gave generally positive results in the liver, with mixed results for the kidney and bone marrow. The end-points that have been evaluated in these studies comprise biomarkers of DNA adducts and various types of chromosomal damage. Tests in bacterial assays gave consistently negative results."

There is a similar conclusion in the IARC report and in the CLH report that glyphosate does not induce **gene mutations** in bacterial assays. In addition, one *in vitro* mammalian cell gene mutation study (Li and Long, 1988) was included in the IARC report whereas three were included in the CLH report, but all were negative.



The *in vivo* **bone marrow tests** are given considerable weight in the IARC mutagenicity evaluation. One chromosomal aberration test (Li and Long, 1988) and three micronucleus tests (Rank, 1993; Bolognesi *et al.*, 1997; Mañas *et al.*, 2009) were included in the IARC report. All four studies were performed with i.p. administration of glyphosate; two were negative and two were positive. Accordingly, the IARC report states that the bone marrow studies gave mixed results. All four studies are also assessed by RAC. RAC finds that deficiencies in design of the study by Mañas *et al.* (2009) renders the biological relevance of the result uncertain, as commented above in the section describing "In vivo studies: Non-human mammalian data". Furthermore, RAC remarks that the micronucleus incidence in the high dose group in the study by Bolognesi *et al.* (1997), is moderate and close to the control frequencies reported for other micronucleus tests. RAC has considered data from 7 additional oral studies and 3 i.p. studies which were all negative and concludes that glyphosate is not mutagenic across the entire range of *in vivo* bone marrow mutagenicity tests.

Studies in exposed humans: The IARC Monograph concluded positive evidence of DNA breakage in blood cells collected from 2 weeks to 2 months after spraying as determined by the Comet assay by Paz-y-Miño *et al.* (2007). However, there was no induction of chromosomal aberrations in blood cells from individuals in 10 communities who were sampled 2 years after the last aerial spraying with a herbicide mix containing glyphosate (Paz-y-Miño *et al.*, 2011), nor an induction of MN in community residents after spraying compared to before aerial spraying with glyphosate-based formulations (Bolognesi *et al.*, 2009). However, IARC remarks that the increase in frequency of micronucleus formation observed immediately after spraying was not consistent with the rates of application used in the regions, and there was no association between self-reported direct contact with pesticide sprays and frequency of binucleated cells with micronuclei.

RAC notes that the results from the human genotoxicity studies are equivocal and that their overall interpretation is challenging due to the time between spraying and blood sampling (from 2 weeks to 2 months), uncertain exposure estimates and the combined exposures to glyphosate and co-formulants and also to other pesticides. RAC concludes that the data available is not sufficient to conclude that glyphosate is the factor likely to explain the association between glyphosate based herbicide and higher incidences of micronuclei in the studies where this has been observed.

Supporting evidence/indicator tests:

IARC, in monograph 112, states that "In-vivo studies in mammals gave generally positive results in the liver, with mixed results for the kidney ...".

RAC notes that two studies (Bolognesi *et al.*, 1997, Mañas *et al.*, 2013) report induction of DNA single strand breaks in liver following either a single i.p or a repeated oral exposure.

Mechanistic studies – oxidative stress:

IARC reported that "there is strong evidence that glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid can act to induce **oxidative stress** based on studies in experimental animals, and in studies in humans *in vitro*. This mechanism has been challenged experimentally by administering antioxidants, which abrogated the effects of glyphosate on oxidative stress. Studies in aquatic species provide additional evidence for glyphosate-induced oxidative stress." On page 69 it states that: "Specifically, it was found that glyphosate induces production of free radicals and oxidative stress in mouse and rat tissues through alteration of antioxidant enzyme activity, depletion of glutathione, and increases in lipid peroxidation. Increases in biomarkers of oxidative stress upon exposure to glyphosate *in vivo* have been observed in blood plasma (Astiz *et al.*, 2009b), liver (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b),



skin (George *et al.*, 2010), kidney (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b), and brain (Astiz *et al.*, 2009b)."

RAC has evaluated the rodent studies with regard to markers of oxidative stress, with the exception of the study by George *et al.* (2010) where dermal exposure to a glyphosate containing formulation showed reduced expression of the antioxidant enzyme (SOD) in skin. RAC considers the study by Astiz *et al.* (2009) to be of uncertain reliability due to deficiencies in the reporting. In addition to the studies evaluated in the IARC report, RAC has included data from the *in vivo* studies by Mañas *et al.* (2009 and 2013) and Dai *et al.* (2016). RAC considers the data from the studies available to be equivocal and concludes that although it appears that glyphosate may induce oxidative stress, this has not been demonstrated in the *in vivo* repeated dose studies suggesting that the effect is weak and of uncertain biological significance.

Comparison with the CLP criteria

The database available for evaluation of germ cell mutagenicity is extensive and includes studies covering bacterial and mammalian cell *in vitro* mutagenicity assays as well as *in vivo* mammalian mutagenicity assays and some human data. The database includes studies of sufficient reliability and relevance to allow a robust evaluation following the requirements of CLP. Mutagenicity data related to exposures to AMPA and glyphosate based herbicide are not considered in this analysis by RAC as the purpose is to provide a harmonised classification of glyphosate itself, the exception being the inclusion of human biomonitoring data. Genotoxicity data from non-mammalian species are not included in the assessment, because the relevance of the findings to humans of such studies conducted using non-standard protocols is less clear than in the many studies available which were conducted using standard protocols and standard animal models, and for the majority of the studies under Good Laboratory Practice.

Category 1A

According to the CLP criteria, classification of a substance as a germ cell mutagen in Category 1A is based on positive evidence from epidemiological studies that the substance induces heritable mutations in germ cells of humans.

A limited number of biomonitoring studies have examined markers of possible genotoxicity in blood cells from humans exposed occupationally or from the general population in regions with high use of glyphosate. Some of these studies showed an apparently positive relationship between exposure to glyphosate and the levels of the markers being studied. However, all these studies were compromised by the lack of clear information about exposure to glyphosate itself and glyphosate-based formulations, and the extent to which other substances or lifestyle factors could have contributed to the findings. In some cases, the low numbers of subjects involved was also a factor. Although not completely negative, these studies do not provide sufficiently robust evidence of glyphosate genotoxicity to justify classification for this endpoint.

The classification of glyphosate as Muta. 1A is not justified.

Category 1B

According to the CLP criteria, classification of a mutagen in Category 1B is largely based on positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations in germ cells.

There was no evidence for mutagenic activity in germ cells of mice or rats at oral doses up to 2000 and 5000 mg/kg, respectively, in the dominant lethal tests presented. However, given that glyphosate has a wide distribution in the body, exposure of germ cells is likely, therefore results from the somatic mutagenicity studies are relevant also for the evaluation of germ cell mutagenicity.

The bacterial mutation assays and mammalian cell gene mutation tests gave consistently negative results. Furthermore, a total of 7 oral and 7 i.p. bone marrow micronucleus tests and two chromosomal aberration test in rodents were reported. All oral tests and three of the i.p. tests were conducted according to OECD TG 474 or 475 and performed according to GLP. The majority of these bone marrow test were negative, but two were positive. One was considered to have deficiencies making the interpretation uncertain and was hence given less weight in the overall assessment. The other presented a statistically significant increase that may well have been within the anticipated control level. Thus, the evidence from these two positive studies does not override the overall conclusion from the numerous other *in vivo* mutagenicity studies, that glyphosate does not induce somatic cell mutations.

The mammalian *in vivo* database is considered sufficient and an overall evaluation indicates that glyphosate does not warrant classification as Muta 1B.

Category 2

Classification in Category 2 is largely based on positive evidence obtained from somatic cell mutagenicity tests in mammals or other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

Glyphosate is only metabolised to a very limited degree and is not a DNA reactive substance. Bacterial and mammalian gene mutation assays were all negative. Thus, the genotoxicity observed for glyphosate in some studies is likely to be caused by indirect mechanisms. Glyphosate appears to induce transient DNA strand breaks as observed in the *in vitro* and *in vivo* Comet assays. However, as glyphosate does not induce gene mutations and bone marrow mutagenicity is considered negative, their biological importance in relation to mutagenicity is equivocal. Further, it is unclear whether oxidative stress is of biological importance as a MoA for glyphosate as the data are equivocal.

Taking all data into account, and based on the overall negative responses in the existing gene mutation and oral mutagenicity tests, RAC concludes that **no classification of glyphosate for germ cell mutagenicity** is warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

In the CLH report, studies using mice and rats as well as epidemiological studies addressing the effects of exposure to glyphosate in humans were assessed. These studies and the findings are discussed in detail below. The main statistical methods used in the animal studies were the Fisher's exact test for pairwise comparisons and the Cochran-Armitage trend test, and in this document these two methods are referred to unless stated otherwise. In their detailed assessment of findings, the DS repeated both the pairwise and trend test statistical calculations for the findings from relevant studies (9 studies in rats and 5 studies in mice; for details, see below).

Rats

The DS noted that they were aware of 9 unpublished long-term feeding studies with the technical active ingredient in rats (summarised in Table 25 of the CLH report) of which 6 were performed in compliance with OECD TG 453. The DS concluded that the remaining three studies (including the studies by Bhide *et al.*, 1997 and Calandra *et al.*, 1974, which were both negative) were "flawed by serious deficiencies", but since tumour data from one of these studies (Lankas, 1981) had been discussed in other assessments, the DS also considered this study in detail in the CLH

report. In addition, the DS briefly summarised two further published studies (in which glyphosate was administered via drinking water), but concluded that these had "strong limitations" and therefore these were not assessed in detail. In one of these (Chruścielska *et al.*, 2000a) a glyphosate (ammonium) salt solution of unknown purity but not the acid was tested and the study was poorly reported, but no evidence of carcinogenicity was observed. In the other study (Séralini *et al.*, 2012), in female animals given a glyphosate formulation, an increased incidence of mammary tumours was seen in females resulting in a shorter lifespan, but the number of animals in each dose group was too small (10/sex/dose) for firm conclusions to be drawn.

The DS noted that the main carcinogenicity findings in rats comprised an increase in islet cell tumours of the pancreas (Stout and Ruecker, 1990; Lankas, 1981), increases in liver tumours and in C-cell adenoma of the thyroid (Stout and Ruecker, 1990), and an increase in interstitial cell tumours of the testis (Lankas, 1981). The DS assessed each of these findings in detail. In the remaining 4 GLP compliant studies in rats conducted according to OECD Guidelines, no increases in tumour incidences were seen.

In the case of the pancreatic tumours, the DS noted that for the low dose males (but not at the two higher doses or in females), when compared pair-wise to the concurrent controls, a re-evaluation of the data confirmed, in the study by Stout and Ruecker (1990; dose range 89-940 mg/kg bw/day) a statistically significant increase in adenomas and in the study by Lankas (1981; dose range 3-31.5 mg/kg bw/d) an increase in adenomas and carcinomas combined. However, the DS also noted a statistically significant positive trend for carcinomas in male animals in the Lankas (1981) study, which had not been previously reported. This was seen in a single affected male at the high dose, but in none of the other animals. There was no incidences of pancreatic tumours in the females. No dose-response relationship was observed and there was no indication of progression to malignant neoplasia in either study. The DS also noted that an increased incidence of pancreatic tumours was not reproducible in other, more recent and OECD TG-compliant studies, in which the incidences of pancreatic cancer in untreated control animals sometimes resembled the incidences reported in these two studies.

The incidences of liver tumours reported by Stout and Ruecker (1990) were re-evaluated by the DS using trend- and pairwise tests. A statistically significant trend was confirmed for the adenomas but no positive trend was observed for the adenoma and carcinoma combined. The DS also noted that a dose-response relationship was "was hardly to be seen" and although absolute and relative liver weights were increased in high dose males in the study, there were no pre-neoplastic findings that might progress to liver tumours.

Increases in the incidence of C-cell adenoma in female rats was seen in the study of Stout and Ruecker (1990) which were negative using a pairwise comparison, but weakly positive in the trend test ($p = 0.0435$). In the absence of such a finding in any of the other rat studies, this increase in C-cell tumours was not considered by the DS to be biologically significant.

An increase of interstitial testicular tumours was observed by Lankas *et al.* (1981). Although there was no clear dose response relationship, at the top dose the difference relative to the control was statistically significant ($p < 0.05$). The DS noted that in the original study report it was argued that the absence of this tumour type in the control group was unusual and that the high dose incidence was "only marginally above the historical control range" and no increase in testicular tumours was observed in any other long-term study with glyphosate in rats, despite much higher doses having been administered.

Mice

The DS summarised and assessed (in table 30 of the CLH report) five OECD TG 451-compliant long-term studies in mice. In two of the studies (Sugimoto, 1997 and Knezevich and Hogan,

1983), high doses greater than 4000 mg/kg bw/day had been administered and the DS noted that there was evidence that the MTD had been exceeded at these doses.

The DS also noted the existence of two further long-term studies in mice, which "did not comply with current standards", in which no increase in any tumour type had been reported, but in which the high dose was considered much too low for a meaningful evaluation. In addition, the DS noted a published study on skin tumour promotion, which was performed with a commercial product that "most likely contains irritating co-formulants" and therefore was not considered to contribute to a decision on the classification of glyphosate. These studies were therefore not assessed.

In the studies assessed, there was evidence of increases in three types of tumours (malignant lymphoma, renal tumours, and haemangiosarcoma; all in males), which were addressed in detail in the CLH report.

Malignant lymphoma was reported in four studies with CD-1 mice, as well as in a study using Swiss mice. The DS assumed that although these were not specifically mentioned in the study by Knezevich and Hogan (1983), these were included in the description of the finding of lymphoreticular neoplasia observed in male CD-1 mice. The DS noted that the statistical significance of the suspected increase in malignant lymphoma in the various studies was very much dependent on the statistical method that is used for data analysis. In the studies by Wood *et al.* (2009) and Sugimoto (1997), the findings were statistically significant when the trend test was applied, but not when a pairwise comparison was performed. The increased incidence in the study of Kumar (2001) was not confirmed either by the trend test or by a different pairwise test but only using the Z-test which had been used in the original study report.

The DS concluded that based on an inconsistent dose response in the individual studies, and a highly variable spontaneous tumour incidence as suggested by the historical control data, it was not likely that glyphosate induced malignant lymphoma in mice. The DS also noted that a possible role of oncogenic viruses should not be ignored. The DS also questioned the human relevance of an effect which was only seen at high doses.

Renal tumours were reported in three studies with CD-1 mice and the study using Swiss mice. A re-evaluation of the histopathological findings from the Knezevich & Hogan (1983) study in CD-1 mice by a Pathology working group (PWG) was conducted.

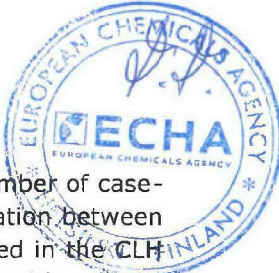
The DS concluded that the renal tumours in mice were not likely to be treatment related, primarily because the incidences of the findings were not statistically significant in comparison with concurrent controls, but also because the incidences at the highest doses were similar to those in controls in other studies, the findings were within the historical control ranges, there were no pre-neoplastic lesions in treated animals and there was no plausible mechanism.

Evidence for development of haemangiosarcoma was seen in male CD-1 mice at the highest dose in 2 studies (Atkinson *et al.*, 1993 and Sugimoto, 1997). The incidences were not statistically significant in comparison with the concurrent controls by a pairwise comparison, but were statistically significant using a trend test. The DS noted that the findings were within the historical control range.

The DS also presented (in table 42 of the CLH report) a summary of the tumour incidences in male CD-1 mice from four studies with glyphosate and the maximum value of the historical control range and concluded that over a wide dose range, there was no evidence of a consistent increase in any tumour type.

Humans

The DS summarised a number of epidemiological studies, including the United States Agricultural Health Study (AHS), which was described as "the largest and most convincing epidemiological



study". The DS noted that some publications arising from the AHS study and a number of case-control studies (which were also summarised) have focused on a possible association between glyphosate exposure and Non-Hodgkin's Lymphoma (NHL) and this was considered in the CLH report in some detail. The DS (in tables 43 and 44 of the CLH report) also considered and compared the evaluations that had been conducted by IARC and the rapporteur member state (Germany) under the pesticide review process on various epidemiological studies.

The DS concluded that overall the epidemiological data did not provide convincing evidence that glyphosate exposure in humans might be related to any cancer type, including NHL. The DS also concluded that epidemiological studies are of limited value for detecting the carcinogenic potential of an active substance in plant protection products "since humans are never exposed to a single compound alone" and the results of the studies are associated with different formulations containing glyphosate or mixtures of different active substances.

Conclusions of the DS

The DS concluded that based on the epidemiological data as well as on data from long-term studies in rats and mice, taking a weight of evidence approach, no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria.

Comments received during public consultation

Most of the large number of comments received during the public consultation addressed carcinogenicity. Comments were received from 9 MSCAs or national government organisations, the remainder being from organisations or individuals.

According to an analysis conducted by the DS, approximately 20% of the general comments contained detailed and scientifically justified arguments, some of which were very extensive. One comment in particular (from an individual) provided extensive comment on the statistical analyses conducted in the CLH report. Published papers accompanied some of the submitted comments.

The DS noted that most of the remaining comments received were variations of standardised text or were general comments concerning the intended use, the risk assessment of glyphosate or further issues without detailed or new toxicological information relevant for hazard identification or on the classification and labelling of glyphosate.

Three comments from the MSCAs indicated general or specific support for the position of the DS for no classification for carcinogenicity. One MSCA provided a critical analysis of the CLH report (including pointing out inconsistencies between the CLH report and the risk assessment report). The remainder provided either cautious or clear support for classification for carcinogenicity in general or for classification in Category 2. In addition, one government authority from Germany (not an MSCA) argued for classification as Carc. 1B.

Comments from Industry agreed with the DS that no classification was warranted. In responding to some of the comments received, the DS indicated that they continued to hold the position that no classification for carcinogenicity was warranted.

In response to a request from the RAC during the accordance check and as a response to several comments received in the public consultation, the DS included an addendum to the CLH dossier in the RCOM, to elaborate further on the weight of evidence related to the three tumour types in mice (renal tumours, malignant lymphoma and haemangiosarcoma). The addendum contained a systematic evaluation according to the IPCS 'Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis' (2001) and was included to further clarify the DS proposal on no classification for carcinogenicity.

The DS addendum consists of two sections:

- (1) Two tables based on Table 52 of the the most recent CLH report template "Compilation of factors to be taken into consideration in the hazard assessment", summarising the available long-term studies with glyphosate in rats (Table 1 of the addendum) and mice (Table 2 of the addendum).
- (2) Systematic evaluation of three tumour types in mice in accordance with the IPCS 'Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis' (2001, TOX2004-2639).

Assessment and comparison with the classification criteria

Non-human data

Seven rat and five mouse carcinogenicity bioassays included in the CLH report form the basis of the current RAC evaluation of carcinogenicity in animals.

RAC also assessed the original full study reports (Robust Study Summaries are included in the RAR). In the original study reports, mostly pairwise comparisons had been made, whereas in the IARC evaluation (2015), trend tests were the preferred statistical tool. The DS re-calculated the statistical significance of the observed tumour incidences by the use of both pairwise comparisons by the Fisher's exact test, and trend analysis by the Cochran-Armitage trend test. RAC presents the *p*-values calculated by the DS in this opinion.

Rat combined chronic toxicity/carcinogenicity studies (see also DS Addendum, Table 1)

Study selection - rat bioassays

Seven long-term studies were available to RAC for the assessment of carcinogenicity in rats following exposure to glyphosate, with six of the studies performed according to OECD TG 453 (Combined Chronic Toxicity/Carcinogenicity Studies). One study, regarded by the DS to have significant reporting deficiencies and insufficient dose levels (Lankas *et al.*, 1981), was included in the carcinogenicity assessment by the DS due to the occurrence of pancreatic and testicular tumours. This study used low doses, thus not satisfying the guideline requirements. A study using adequate dose levels has subsequently been performed (Stout and Ruecker, 1990).

The DS found the following studies not suitable for evaluation of classification and these were not considered in detail in the overall RAC evaluation: Bhide (1997); Calandra (1974); Chruścielska *et al.* (2000); Séralini *et al.* (2012). The studies by Bhide *et al.* (1997) and Calandra *et al.* (1974) were negative.

The study by Séralini *et al.* (2012) was considered to be inadequate for the evaluation of glyphosate carcinogenicity also by the IARC working group. The IARC working group also stated that the study by Chruścielska *et al.* (2000) had limited information, and that no significant increase in tumour incidences was reported. The IARC report included the studies by Brammer (2001), Atkinson (1993), Stout and Ruecker (1990) and Lankas (1981), but not the studies by Wood (2009), Enomoto (1997) and Suresh (1996).

According to the DS, no evidence of carcinogenicity was observed in the long-term rat studies after an evaluation of all data. IARC stated that there were no increases in tumour incidences in the glyphosate treated groups in the studies by Atkinson (1993) and Brammer (2001). However, IARC pointed out a significant increase in the incidence of pancreatic islet cell adenoma in males in two Sprague-Dawley rat studies (Lankas 1981; Stout and Ruecker 1990) and that the latter study also showed a significant positive trend in the incidences of hepatocellular adenoma in males and of thyroid C-cell adenoma in females.

RAC has evaluated the neoplasias of the rat pancreas, liver and thyroid based on data provided in the CLH report and the RAR.

The suggestion of increased incidences in tumours of the pancreas, liver and thyroid are mainly based on findings in the study by Stout and Ruecker (1990), with support for pancreatic tumours also from the study by Lankas (1981). There were no significant effects on body weight noted in males of any dose group in the study by Stout and Ruecker (1990). In high-dose females, body weights were statistically significantly reduced from week 7 to approximately the 20th month.

Pancreatic islet cell tumours

In the table below, the incidences of pancreatic islet cell tumours in male rats in all 7 studies are shown.

Incidences of pancreatic islet cell adenomas and carcinomas combined in male rats

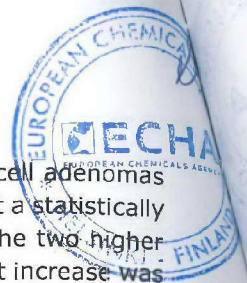
Study (strain)	Control	Low dose	Mid dose	Second mid dose	High dose	Response Fisher's exact test
Wood <i>et al.</i> , 2009 (Wistar)	4 / 51 (7.8%)	1 / 51 (86 mg/kg bw/d)	2 / 51 (285 mg/kg bw/d)	-	1 / 51 (1077 mg/kg bw/d)	No significant increase
Brammer <i>et al.</i> , 2001 (Wistar)	1 / 53 (1.9%)	2 / 53 (121 mg/kg bw/d)	0 / 53 (361 mg/kg bw/d)	-	1 / 52 (1214 mg/kg bw/d)	No significant increase
Enomoto, 1997 (Sprague-Dawley)	4 / 50 (8.0%)	1 / 50 (104 mg/kg bw/d)	2* / 50 (354 mg/kg bw/d)	-	1 / 50 (1127 mg/kg bw/d)	No significant increase
Suresh, 1996 (Wistar)	3 / 48 (6.3%)	0 / 30 (6.3 mg/kg bw/d)	0 / 32 (59.4 mg/kg bw/d)	-	1 / 49 (595.2 mg/kg bw/d)	No significant increase
Atkinson <i>et al.</i> , 1993 (Sprague-Dawley)	7 / 50 (14.0%)	1 / 24 (10 mg/kg bw/d)	2 / 17 (100 mg/kg bw/d)	2 / 21 (300 mg/kg bw/d)	1 / 49 (1000 mg/kg bw/d)	No significant increase
Stout and Ruecker, 1990 (Sprague-Dawley)	2* / 43 (4.7%)	8 / 45 (17.8%) (89 mg/kg bw/d)	5 / 49 (10.2%) (362 mg/kg bw/d)		7 / 48 (14.6%) (940 mg/kg bw/d)	Significant increase in adenoma in low dose vs control
Lankas, 1981 (Sprague-Dawley)	0 / 50 (0.0%)	5 / 49 (10.2%) (3 mg/kg bw/d)	2 / 50 (4%) (10.3 mg/kg bw/d)	-	3* / 50 (6%) (31.5 mg/kg bw/d)	Significant increase in adenoma in low dose vs control

*Including one carcinoma

Two of the seven studies show an increase in pancreatic adenomas (Stout and Ruecker, 1990; Lankas, 1981).

In the study by Stout and Ruecker (1990) an increase in pancreatic islet cell adenomas was reported, but the increase did not reach statistical significance when using the Cochran-Armitage trend test. The pairwise Fisher's exact test was only positive for the low dose group compared to control. Further, there was no progression to malignancy in the exposed groups since the only carcinoma was reported in the control group. In this study no pancreatic islet cell carcinomas were reported in females and the adenoma incidences (5/60, 1/60, 4/60, and 0/59) did not show an increase in exposed groups versus controls. There were no dose-related increases in pancreatic hyperplasias in male or female rats suggesting that the adenomas were spontaneous and not treatment related.

According to the RAR, the incidence of adenomas in low-dose males (17.8%), mid-dose males (10.2%) and high-dose males (14.6%) was outside the historical control range (1.8 – 8.5 %) for this laboratory.



In the study by Lankas (1981) no clear dose-related increase in pancreatic islet cell adenomas and carcinomas was reported. However, when using the pairwise Fisher's exact test a statistically significant increase in adenoma was reported in the low dose group, but not in the two higher dose-groups. When using the Cochran-Armitage trend test a statistically significant increase was found for carcinomas ($p=0.046$), but not for adenomas. Only low doses were administered in this study.

The elevated incidences of pancreatic adenomas observed in glyphosate exposed groups in the two studies discussed above were only observed in males and did not show a dose-response relationship. Furthermore, they were not supported by findings in the additional five long-term guideline studies in rats (Table above) in which no increase in pancreatic islet cell tumours were reported in response to glyphosate. In four of these studies, the incidences were higher in the control groups than in the glyphosate exposed groups. The findings do not seem to be strain dependent as the two other studies in Sprague-Dawley did not show any increases in pancreatic islet cell tumours.

Liver tumours

Liver adenomas and carcinomas in male rats in the Stout and Ruecker (1990) study

Dose (mg/kg bw/d)	Male rats	Liver adenoma	Liver adenoma + carcinoma
0	44	2	5
89	45	2 (1.000)	4 (0.739)
362	49	3 (1.000)	4 (0.732)
940	48	7 (0.162)	9 (0.392)
Cochran-Armitage Trend test (p-value)		0.0171	0.0752

p-values in brackets when using Fisher's exact test.

A positive trend for liver adenomas was reported in the study by Stout and Ruecker (1990) in male rats (Table above). The increase in adenomas was statistically significant when using the Cochran-Armitage trend-test, but not in the pairwise testing against controls (Fisher's exact test). There was no progression to malignancy in the exposed groups as the incidence of liver carcinomas was slightly higher in controls than in the glyphosate treated groups. No statistically significant increase was reported for liver adenomas and carcinomas combined.

At the interim sacrifice, relative liver weights were slightly, but statistically significantly increased in high-dose males whereas absolute and relative liver weight was increased in high dose males at the end of the study. No pre-neoplastic liver lesions were reported in the CLH report or the RAR.

The hepatocellular adenoma incidences in the glyphosate treated animals were within the historical control range from the test facility (1.4%-18.3%) as cited by EPA (EPA 2015).

No significant increases in glyphosate-related liver tumours were reported in the other long-term studies in rats.

Thyroid C-cell tumours

Thyroid C-cell adenomas and carcinomas in study by Stout and Ruecker (1990)

Dose (mg/kg bw/d)	Female rats Adenomas; Carcinomas	Fisher's exact test	Male rats Adenomas/ Carcinomas
0	2/57 (3.5%);		2/54 (3.7%);



Dose (mg/kg bw/d)	Female rats Adenomas; Carcinomas	Fisher's exact test	Male rats Adenomas/ Carcinomas
	0/57		0/54
89	2/60 (3.3%); 0/60	NS	4/55 (7.3%); 2/55
362	6/59 (10.2%); 1/59 (1.7%)	NS	8/58 (13.8%); 0/58
940	6/55 (10.9%); 0/55	NS	7/58 (12.1%); 1/58
Cochran- Armitage Trend test (p-value)	p=0.0435 (adenomas)		Non-significant

An increase in the incidence of thyroid C-cell adenomas was reported for both sexes in the study by Stout and Ruecker (1990) and a significant trend was found for female rats using the Cochran-Armitage test with a p-value of 0.0435. No statistical significance was found when using pairwise comparison (Fisher's exact test). For males, the increased incidences of adenomas or combined adenomas/carcinomas were not statistically significant. No progression from adenoma to carcinoma is indicated in this study.

The thyroid C-cell adenoma incidences in the high dose glyphosate treated animals were slightly higher than the historical control range (3.3%-10.0% in females) as cited by EPA (2015).

No increase in thyroid C-cell adenomas was reported in the other long-term studies in rats. In these other studies, there were no increases in pre-neoplastic histological lesions and no thyroid weight change was noted in response to glyphosate exposure.

Summary of rat long-term/carcinogenicity studies:

Seven rat combined chronic toxicity/carcinogenesis studies are included in the RAC evaluation. Six of these studies are regarded as valid since they are guideline compliant studies and used sufficiently high doses and sufficient numbers of animals per dose group. The study by Lankas (1981), a low-dose study with important reporting deficiencies, is included in the opinion as a supporting study for the evaluation of potential increases in pancreatic adenomas. No treatment-related reductions in survival were observed in the rat studies. Based mainly on information provided in the CLH report and the RAR, RAC has evaluated data related to tumours in the pancreas, liver and thyroid.

In male rats, increased incidences of benign pancreatic and liver tumours was reported in the study by Stout and Ruecker (1990) with some support for pancreatic islet cell adenoma from the study by Lankas (1981). The increase in pancreatic islet cell adenoma was significant in a pairwise testing of the low dose group compared with the control group, but not in the trend test. The increases in liver adenomas were not significant in the pairwise testing, but were positive in the trend test (p=0.0171). Stout and Ruecker (1990) reported an increase in thyroid C-cell adenoma in males and females. The increased incidences were not significant in males, and were only statistically significant in the trend test in females (p=0.0435) and not in pairwise testing versus control.

The significant tumour incidence increases were only observed for benign neoplastic lesions (adenomas) and no progression into more malignant forms were observed for any of the tumour types evaluated. Furthermore, increased incidences of the pancreatic islet adenomas and the hepatocellular adenomas were only observed in male rats.



The incidences of pancreatic islet adenomas were above the historical control range from the test facility, whereas the liver adenoma incidences were within the historical control range and those for the thyroid C-cell adenoma were at the upper range of the historical control data.

Limited information was provided to RAC on potential findings in the planned interim sacrificed animals.

No significant treatment related increases in these tumours were observed in the five more recent guideline studies. The general lack of increases in pre-neoplastic lesions in the affected organs as well as a lack of progression toward increased malignancy, suggest that the findings in the study by Stout and Ruecker (1990) is sporadic in nature. This is further supported by lack of consistency between males and females for pancreatic and liver tumours and the negative findings in the five more recent rat cancer bioassays.

RAC considers that the rat studies did not demonstrate convincing evidence of glyphosate induced neoplasia across the seven studies evaluated and therefore did not support classification for carcinogenicity.

Mouse carcinogenicity studies (see also DS Addendum, Table 2)

Study selection - mouse bioassays

Five long-term studies in mice were available to RAC for the assessment of carcinogenicity following exposure to glyphosate, all performed according to OECD TG 451 with four studies in CD-1 mice and one study in Swiss albino mice. In none of the studies with CD-1 mice was glyphosate treatment associated with reduced survival. There was a slightly higher mortality in the Swiss albino mice of the high dose group in both males and females.

Three mouse carcinogenicity studies were included in the IARC report. These were the studies by Knezevich and Hogan (1983), Atkinson *et al.* (1993) and a dermal initiation-promotion study by George *et al.* (2010). The latter study used exposure to a glyphosate based herbicide and is therefore not evaluated in the current RAC opinion. The following three mouse studies evaluated by RAC were not evaluated by IARC: Sugimoto (1997); Wood *et al.* (2009); Kumar *et al.* (2001).

The following tumour types were evaluated by RAC: renal tumours, haemangiosarcomas and malignant lymphomas. The RAC evaluation of the mouse cancer studies is mainly based on information provided in the CLH report and the RAR (including full access to the original study reports).

Renal neoplasms:

Incidences of renal adenomas and carcinomas combined in male mice

Study (strain)	Control	Low dose	Mid dose	High dose	Fisher's exact test (high dose vs control) Cochran-Armitage trend test
Knezevich and Hogan ^a 1983; CD-1	1 / 49 (2%)	0 / 49 (157 mg/kg bw/d)	1# / 50 (2%) (814mg/kg bw/d)	3## / 50 (6%) (4841 mg/kg bw/d)	p= 0.617 p=0.0339
Atkinson <i>et al.</i> , 1993 CD-1	2# / 50 (4%)	2# / 50 (4%) (100 mg/kg bw/d)	0 / 50 (300 mg/kg bw/d)	0 / 50 (1000 mg/kg bw/d)	No significant increase
Sugimoto, 1997 CD-1	0 / 50	0 / 50 (165 mg/kg bw/d)	0 / 50 (838 mg/kg bw/d)	2 / 50 (4%) (4348 mg/kg bw/d)	p= 0.495 p=0.0078
Wood <i>et al.</i> , 2009 CD-1	0 / 51	0 / 51 (71 mg/kg bw/d)	0 / 51 (234 mg/kg bw/d)	0 / 51 (810 mg/kg bw/d)	No significant increase



Study (strain)	Control	Low dose	Mid dose	High dose	Fisher's exact test (high dose vs control) Cochran-Armitage trend test
Kumar <i>et al.</i> , 2001 Swiss albino	0 / 50	0 / 50 (15 mg/kg bw/d)	1 / 50 (151 mg/kg bw/d)	2 / 50 (4%) (1460 mg/kg bw/d)	p = 0.495 p = 0.039

^aPWG re-evaluation of kidney lesions, #including one carcinoma, ##including two carcinoma

As noted by the pathology working group (PWG) in their re-evaluation of the data in the Knezevich and Hogan study (1983), differentiation between tubular-cell adenoma and tubular-cell carcinoma is not always clearly apparent and both lesions are derived from the same cell type. Accordingly, it is the combined incidences that have been used in the statistical analysis.

Low, but elevated incidences of renal tumours were reported at the high doses exposures in three of the five mouse carcinogenicity studies (Table above). The increases in renal tumours were not statistically significant in pairwise comparisons (Fisher's exact test), but when the Cochran-Armitage trend-test was used, statistical significance was reported in these studies.

All kidney tumours were observed at termination.

No increase was reported in related preneoplastic lesions (renal tubular hyperplasia or necrosis) in male mice. In the study by Knezewitch and Hogan (1983), non-neoplastic kidney pathology in the form of chronic interstitial nephritis was reported to be increased, but is not considered to be a precursor for renal tubular cell adenoma.

Renal adenomas and carcinomas are rare tumours in CD-1 mice. Spontaneous control incidences for CD-1 male mice obtained from Charles River Laboratories report a mean incidence of 0.24 and a range of 0-4% for adenoma and a mean incidence of 0.14 and a range of 0-2% for carcinoma from studies initiated between 1987 and 2000 (Giknis and Clifford, 2005, ASB2007-5200). The incidences in the high dose CD-1 mice are at the upper end or slightly outside the control range for renal adenomas/carcinomas. Historical control data from the test facility (as cited in the EPA report, 2015) for the Knezewitch and Hogan (1983) study, had a range between 0 and 3.3%. No historical control data were available to RAC for renal tumours from the test facilities for the Sugimoto (1997) or Kumar (2001) studies.

In two of the five studies, no renal tumours were reported at the two highest doses and in two studies, adenomas/carcinomas were reported in the control groups. Furthermore, no increase in renal tumours was reported in female mice. There was a positive trend in male mice, but the findings were not consistent across all studies. RAC notes that although the *p*-value determined in the trend test in the study by Sugimoto (1997) indicated that the finding was statistically significant, there were only two adenomas among the 200 males examined in this study.

In two of the three positive studies (Sugimoto *et al.*, 1997 and Knezewitch and Hogan, 1983), increased tumour incidences were only observed at very high doses (>4000 mg/kg bw/d) at which the body weight gain in males were decreased compared to controls by up to 11% and 15% in the Knezevich and Hogan (1983) and the Sugimoto (1997) study, respectively. The OECD TG 451 for carcinogenicity studies does not give a precise top dose recommendation, but states that the highest dose level should elicit signs of minimal toxicity, with depression of body weight gain of less than 10%. RAC therefore gives less weight to the findings at these very high dose levels. The human relevance of the renal tumours at very high doses is considered to be low and the overall evidence for the increase in renal tumours having been caused by glyphosate is considered insufficient for classification.



Haemangiosarcoma

An increased incidence of haemangiosarcoma was reported in two studies in CD-1 mice (see the table below).

Incidence of haemangiosarcomas in male CD-1 mice

Dose (mg/kg bw/d)	Haemangio-sarcoma	Fisher's exact test	Dose (mg/kg bw/d)	Haemangio-sarcoma	Fisher's exact test
Atkinson <i>et al.</i> , 1993 (24 months)			Sugimoto, 1997 (18 months)		
0	0 /50		0	0 /50	
100	0/50		165	0 /50	
300	0 /50		838	0/50	
1000	4/50 (8%)	p=0.059	4348	2/50 (4%)	p=0.495
Cochran-Armitage trend test	p=0.0004			p=0.0078	

Haemangiosarcomas are vascular tumours and they were mostly found in liver and spleen. Increased incidences of haemangiomas were reported in high dose animals in the studies by Atkinson *et al.* (1993) and Sugimoto (1997). The incidence in the high dose male mice in the Atkinson *et al.* (1993) study was at the upper edge (8%) of the historical control data of the performing laboratory (mean incidence at 3%, range 0-8%). No historical control data for haemangiosarcoma from the Sugimoto (1997) test facility was available to RAC. The 4% incidence at the high dose (greater than 4000 mg/kg bw/d) in the Sugimoto (1997) study is within the historical control range for CD-1 mice obtained from Charles River Laboratories with a mean incidence of 0.99% and a range of 0-12% (Giknis and Clifford, 2005, ASB2007-5200).

When pairwise comparison with the Fisher's exact test was used, the increase in haemangiosarcomas reported in the study by Sugimoto (1997) was not statistically significant. However, when the Cochran-Armitage trend-test was used statistical significance was reported in both studies. RAC notes that although the p-value determined by the trend test in the study by Sugimoto (1997) indicated that the finding was statistically significant, there were only two tumours among the 200 males examined.

In three of the five studies, no increases in the incidences of haemangiosarcomas were reported in response to glyphosate treatment. Female mice had variable, but low incidences in haemangiosarcomas, with no apparent dose-response relationships. Across both sexes and all five studies, the findings of an increase in haemangiosarcomas in response to glyphosate exposure were inconsistent and the incidences are considered to be within the historical control range.

Malignant lymphoma

In mice, lymphoma is a common, spontaneously occurring neoplasm. An increased incidence of malignant lymphoma was reported in three carcinogenicity studies in CD-1 mice and one study in Swiss albino mice (see the table below).

Incidences of malignant lymphoma in male and female mice



Study; Strain; Duration		Males				Females			
Wood <i>et al.</i> , 2009; Crj:CD-1; 18 months	Dose (mg/kg bw/d)	0	71	234	810	0	98	299	1081
	Affected	0/51	1/51 (2%)	2/51 (4%)	5/51 (10%)	11/51	8/51	10/51	11/51
	Fisher's exact test				$p = 0.056$	No significant increase			
	Cochran-Armitage trend test	$p = 0.0037$							
Sugimoto, 1997; Crj:CD-1; 18 months	Dose (mg/kg bw/d)	0	165	838	4348	0	153	787	4116
	Affected	2/50 (4%)	2/50 (4%)	0/50	6/50 (12%)	6/50	4/50	8/50	7/50
	Fisher's exact test				$p = 0.269$	No significant increase			
	Cochran-Armitage trend test	$p = 0.0085$							
Atkinson <i>et al.</i> , 1993; CD-1 (sub-strain not specified); 24 months	Dose (mg/kg bw/d)	0	100	300	1000	0	100	300	1000
	Affected [#]	4/50 (8%)	2/50 (4%)	1/50 (2%)	6/50 (12%)	14/50	12/50	9/50	13/50
	Fisher's exact test				$p = 0.741$	No significant increase			
	Cochran-Armitage trend test	$p = 0.076$							
Knezevich and Hogan ^a 1983; Crj:CD-1; 24 months	Dose (mg/kg bw/d)	0	157	814	4841	0	190	955	5874
	Affected	2/48 (4%)	5/49 (10%)	4/50 (8%)	2/49 (4%)	6/50 (12%)	6/48 (13%)	7/49 (14%)	11/49 (22%)
	Fisher's exact test	No significant increase				No significant increase			
	Cochran-Armitage trend test	No significant increase				No significant increase			
Kumar <i>et al.</i> , 2001; Swiss albino	Dose (mg/kg bw/d)	0	15	151	1460	0	15	151	1460
	Affected	10/50 (20%)	15/50 (30%)	16/50 (32%)	19/50 (38%)	18/50	20/50	19/50	25/50 (50%)
	Fisher's exact test				$p = 0.077$				$p = 0.225$
	Cochran-Armitage trend test	$p = 0.065$				$p = 0.068$			

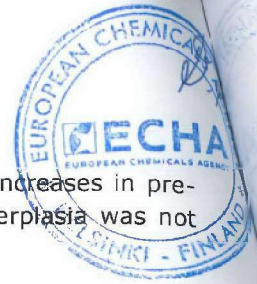
[#] based on histological examination of lymph nodes with macroscopic changes.

^alymphoreticular neoplasms (total); malignant lymphoma not used as a separate entity.

When pairwise comparison with Fisher's exact test was used, the increases in lymphomas did not reach statistical significance in any of the studies. In two of the studies in CD-1 mice (Sugimoto, 1997; Wood *et al.*, 2009), a statistically significant trend for malignant lymphoma was observed in male animals when using the Cochran-Armitage trend test.

No significant increases in malignant lymphomas were found in the study by Knezevich and Hogan (1983). In this study, malignant lymphoma was not used as a separate histopathological entity. However, the term "lymphoreticular neoplasms" is considered to include the group of malignant lymphomas and the findings were reported to be non-significant in the RAR.

The tumour incidence of 12% at the high dose of 4348 mg/kg bw/d in the study by Sugimoto (1997) was within the relevant historical control range for Crj:CD-1 male mice obtained from the laboratory in which the study was performed (mean 6.3%; range of 3.9% - 19.2%, the majority of the studies had a control incidence $\leq 6\%$, 9 studies initiated between 1993 to 1998; Kitazawa,



2013, ASB2014-9146). In the study by Sugimoto (1997), treatment related increases in pre-neoplastic lymph node pathology in the form of mesenteric lymph node hyperplasia was not reported.

The 10% incidence in the study by Wood *et al.* (2009) was borderline significant in the pairwise Fisher's exact test. However, the incidence of lymphomas in controls is very low and there are limited historical control data available from the laboratory. The only information provided to RAC regarding control data from the same laboratory as Wood *et al.* (2009) was from a study performed in 2008 with an incidence of malignant lymphoma in the control group at 12% (in males and females). Further, control incidences for malignant lymphomas in male CD-1 mice from a control database of the Harlan Laboratories between 2000 – 2010 had a mean of 7.5% with a range of 0 – 32% (Letter from Eric Wood, 2010). The data provided is for 24-month and not 18-month studies and appears to be from different test facilities. The incidence of malignant lymphomas has a strong age component and thus the range given is not considered representative for the 18-month Wood (2009) study. RAC has also included control incidences for Crl:CD-1 mice obtained from Charles River Laboratories (mean incidence in males of 2.7% and a range of 0-14% for the 18-month studies; Giknis and Clifford, 2005, with studies initiated between 1987 - 2000, ASB2007-5200). In the RAR, a second report from Giknis and Clifford (2010) is mentioned describing control tumour incidences in CD-1 mice in studies initiated in the period between 2002-2006 (mean 2.5%; range 0-6.7% in males from 8 studies of 18 months duration). It should be noted that these control data are from different laboratories and should thus be used with caution. It appears from the available control data that the incidences of malignant lymphomas in Charles River CD-1 mice are relatively variable and the incidences reported in the study by Wood (2009) is considered to be within or slightly above reported control values. No treatment related increases in non-neoplastic lesions such as lymph node hyperplasia were reported in this study.

There was no significant increase in malignant lymphomas in the study by Atkinson (1993). It should be noted that only those lymph nodes which showed macroscopic changes were investigated histologically. This may lead to an underestimation of the actual tumour numbers. In this study, no treatment related increases in non-neoplastic lymph node pathology in the form of mesenteric lymph node hyperplasia was found in the animals examined. No historical control data from the test facility was identified. RAC has used historical control incidences for CD-1 mice obtained from Charles River Laboratories (mean incidence in males of 5.3 % and a range of 0-21.7 % for the 24-month studies; Giknis and Clifford, 2005, with studies initiated between 1987-2000, ASB2007-5200). It should be noted that the substrain of CD-1 mice used in the study by Atkinson (1993) is not known and the data should be used with caution.

In Swiss albino mice (Kumar *et al.*, 2001) the incidence of malignant lymphoma in male and female mice at the top dose was 38% and 50%, respectively. However, the high background incidence in this strain must be taken into consideration. The historical control data, according to information in the study report (no additional information given on the basis of these historical control data), was in males a mean of 18.4% with a range of 6-30% and in females a mean of 41.6 with a range of 14-58%. Thus, the incidences of malignant lymphomas were above the upper range of the historical control data for the male mice.

No significant increases in malignant lymphomas were found in the mouse studies when assessed by the pairwise Fisher's exact test. However, in two of the five studies, a significant positive trend for malignant lymphoma incidences in males was reported. In two studies, increases were observed that were not statistically significant. In the fifth and oldest of the studies, the term malignant lymphoma was not used, but there was no statistically significant increase in lymphoreticular neoplasms reported in this study in response to glyphosate exposure. Thus, the lymphoma incidences in male mice show a slight, but clearly variable increase. Further, no

increase in treatment related non-neoplastic lymph nodes were reported, thus supporting the conclusion that the tumours were of a spontaneous nature. The biological and human relevance of the findings is uncertain for the following reasons:

- i) the maximum incidences were regarded to be within the historical control range for the CD-1 mice, although adequate historical control data were not available for all studies;
- ii) the increases in malignant lymphoma incidences appeared to be confined to the high dose groups in the CD-1 mice;
- iii) the incidence of malignant lymphomas is known to be related to the age of the animals. However, significant associations between exposure to glyphosate and induction of malignant lymphomas were not observed in the 24-month studies. Furthermore, there was no reduction in overall survival in the exposed groups;
- iv) no parallel increases were observed in female CD-1 mice. It is known that female CD-1 mice are usually more prone to develop spontaneous malignant lymphoma than male mice (Son and Gopinath, 2004, ASB2015-2533). The lymphoma incidences were generally higher in females than in males, but no glyphosate related increases were seen in female CD-1 mice.

Summary of mouse carcinogenicity studies

Five mouse carcinogenicity studies are included in the RAC evaluation. All these studies are regarded as valid because they are considered to be guideline compliant (four are also GLP compliant) and all used sufficiently high doses and sufficient number of animals. No treatment-related reductions in survival were observed in these studies. Based mainly on information provided in the CLH report and the RAR, RAC has evaluated data related to kidney tumours, haemangiosarcomas and malignant lymphomas.

An increase in renal neoplasms (adenomas and carcinomas combined) was reported in males at the top doses in three of the five studies. Furthermore, an increase in haemangiosarcoma was reported in CD-1 males at the top doses in two of the studies, and an increased incidence of malignant lymphoma was reported in three carcinogenicity studies in CD-1 mice and one study in Swiss albino mice.

The observed increases in tumour incidences were all non-significant in pair wise comparisons with control groups by the Fisher's exact test. However, several of the findings were positive when tested using the Cochran-Armitage trend test. In two of the studies (Kumar 2001; Sugimoto, 1997), tumours were observed at multiple sites in males in the top dose groups.

All tumours were observed at termination and RAC has no information concerning any possible reduction in tumour latency. However, for the renal adenomas there was no evidence for a progression to malignancy in two of the studies, whereas the data for the third study (Knezevich, 1983) was equivocal.

The high dose levels in two of the five mouse studies (Sugimoto 1997; Knezevich and Hogan, 1983) exceeded 4000 mg/kg bw/d and the body weight gain in males in the high dose group was decreased by more than 15% compared to controls in the Sugimoto (1997) study suggesting that the doses used were excessive and exceeded the MTD (OECD TG 451 and 116)¹. The biological relevance of the slight increases in tumours in these two studies are considered equivocal since they were seen only at the top doses.

¹ According to OECD 451 the maximum dose should result in a "depression of body weight gain (approximately 10%)". Also according to the IUPAC Gold Book, from 1997, current test guidelines (OECD, EPA, EU and JMAFF) for long-term studies state that the highest dose tested should be at the maximum tolerated dose (MTD), conventionally interpreted as a dose causing non-lethal toxicity, often noted as reduced body weight gain of 10% or more.

In mice, the incidences of renal neoplasm and haemangiosarcomas were increased only in males. Malignant lymphoma was present in both male and female mice reflecting that this is a very common spontaneous neoplasm in mice. However, only in the Swiss albino mice a glyphosate-associated increase in this tumour type in females was observed. There is no toxicokinetic data to RAC's knowledge in support of significant differences in ADME between male and female mice, thus the mostly negative findings in female CD-1 mice is regarded as a sign of low consistency of the mouse carcinogenicity data.

All the five studies report a positive trend in males for one or more of the tumour types evaluated suggesting a potential concern for a tumour effect at high glyphosate doses. However, in the cases where increased tumour incidences were found in the high dose groups, the incidences were either within or slightly above the range of historical control data or spontaneous incidence levels reported for CD-1 mice. Furthermore, the apparent sex differences in response remain unexplained and this lowers the consistency of the reported findings in mice. The increased tumour incidences observed is therefore considered to be of equivocal biological relevance. .

A number of organisations, international (WHO/JMPPR), EU (EFSA) and national (for example US EPA, Australian APVMA) have assessed, or are in the process of assessing, the carcinogenic potential of glyphosate. So far, only IARC has concluded that glyphosate is carcinogenic (and genotoxic). Therefore a detailed comparison of the carcinogenicity evaluation conducted by IARC and RAC is provided below.

Comparison with the IARC evaluation

There is a high degree of similarity between the IARC and the CLP criteria for carcinogenicity classification. However, under the CLP Regulation, where the criteria cannot be applied directly to available identified information, there is an obligation to "... carry out an evaluation by applying a weight of evidence determination using expert judgement ...", which involves "... weighing all available information having a bearing on the determination of the hazards of the substance ...".

IARC (monograph 112) states in their rationale for classifying glyphosate in Group 2A: "In addition to limited evidence for the carcinogenicity of glyphosate in humans sufficient evidence for the carcinogenicity of glyphosate in experimental animals, there is sufficient evidence in animals for carcinogenicity of glyphosate".

The definition of sufficient evidence of carcinogenicity (common to both IARC and CLP) is that: "a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;"

The IARC monograph states, concerning the studies in rats: "For the five feeding studies in rats, two studies in the Sprague-Dawley strain showed a significant increase in the incidence of pancreatic islet cell adenoma in males - one of these two studies also showed a significant positive trend in the incidences of hepatocellular adenoma in males and of thyroid C-cell adenoma in females. Two studies (one in Sprague-Dawley rats, one in Wistar rats) found no significant increase in tumour incidence at any site."

The IARC monograph states, concerning the studies in mice: "There was a positive trend in the incidence of renal tubule carcinoma and of renal tubule adenoma or carcinoma (combined) in males in one feeding study in CD-1 mice. Renal tubule carcinoma is a rare tumour in this strain

mice. No significant increase in tumour incidence was seen in female mice in this study. In the and feeding study, there was a significant positive trend in the incidence of angiosarcoma in male CD-1 mice. No significant increase in tumour incidence was seen in female mice in this study."

It is noted that the evaluation performed by RAC is based on a larger experimental database than the IARC evaluation as presented in the CLH report (9 vs 5 rat studies and 5 vs 2 mouse studies, respectively).

In contrast to IARC, RAC does not consider that a genotoxic MoA has been demonstrated for glyphosate (see preceding section on Germ cell mutagenicity).

Human data – epidemiological studies

In the epidemiological studies described below, the data relates to exposure to glyphosate based herbicide, not specifically to glyphosate. An overview table (see Tables 43 to 45 of the CLH report) of the epidemiological studies assessed by IARC is available in the CLH report and in the RAR, and is not reproduced here. Many of the studies are interlinked and are used in the reviews, meta-analyses etc. An overview of the relationship between the most relevant studies are given in the table in annex 3 of this opinion. Some additional publications were brought forward in the public consultation and are listed below. RAC notes that exposure to Roundup® – a glyphosate based herbicide - has occurred in agriculture since 1974 (U.S.), and later to other glyphosate based herbicides. The use of glyphosate increased massively, especially in the U.S. after the introduction of genetically modified glyphosate-tolerant crops in 1996.

Available epidemiological studies generally consist of **cohort** studies and **case-control** studies¹ on cancer, as well as reviews, re-analyses/pooled analyses, systematic reviews and meta-analyses of the aforementioned studies. No other source of human data is available apart from epidemiological studies. Findings of non hodgkins lymphomas (NHL) is of particular interest in the CLH report and in focus in this opinion, but other lymphomas and leukemias, and other cancer types have also been studied. RAC notes that NHL is not a specific disease but a broad spectrum of disorders more correctly referred to as lymphocytic lymphomas, each with possible different aetiologies. They are all classified as not being Hodgkin's lymphoma, and the terminology has changed over the years - some lymphomas are described differently today compared to previously. This complicates the evaluation of the studies.

Cohort study

The U.S. Agricultural Health Study (AHS)

A single large prospective cohort study is available – the U.S. Agricultural Health Study (AHS), which enrolled 57311 private and commercial applicators (farmers/registered pesticide applicators, and in addition spouses and children, in total 75000 participants from Iowa and North Carolina) (De Roos *et al.*, 2005). The study was initiated by the National Cancer Institute (NCI) in cooperation with the National Institute of Environmental Health Sciences (NIEHS), National Institute for Occupational Safety and Health (NIOSH) and EPA. The study design was first described by Alavanja *et al.* (1996), later reported by De Roos *et al.* (2005), and the study is still ongoing. The exposure assessment was initially planned to be based on interviews and questionnaires (e.g. on frequency – days of use of pesticides/year - and duration – years of use of pesticides) but also on actual measurements of exposure / environmental and biological

¹ In cohort studies the people are prospectively followed and with a view to determining whether those exposed to a substance develop a disease more frequently than those who have not been exposed. In a case-control study, the exposure in cases in which people have a particular disease are compared retrospectively with those who do not have the disease. In both cases the intention is to establish whether exposure has had a role in development of the disease.

monitoring (in 200 families in the cohort). The AHS was evaluated by IARC to be the only cohort study to date to have published findings on exposure to pesticides and the risk of cancer at many different sites. Several additional epidemiological analyses, such as nested case-control studies, have been carried out and published based on this cohort. Even if the number of participants in the AHS is large, it would have had to be even larger in order to contribute a sufficient number of cases of rare cancers, such as multiple myeloma (MM, 32 cases found) to obtain significant results. There were 92 cases of NHL after a follow-up time of 6-7 years which did not identify an increased risk, as described below. Age, smoking, other pesticides, alcohol consumption, family history of cancer and education were considered as potential confounders by De Roos *et al.* (2005). RAC notes that the individual exposure time is longer than the follow-up time, as the exposure probably preceded the start of the study (no information reported on actual exposure length or latency time from start of exposure to end of follow-up). The cancer cases, such as NHL, were identified as soon as possible after diagnosis and investigated using nested case-control studies¹.

The strengths of this prospective cohort study are that the collection of exposure information was done at the start of follow-up (thus independent of health status in order to avoid recall bias), the control of confounders like the use of other pesticides, even investigating the exposure-response relationship and the absence of any proxy respondents. However due to the short follow-up time the numbers of cases were relatively low for many cancer types, which results in wide confidence intervals for the observed risk estimates.

Case-control studies

Other study populations

There are also other populations besides the one contained in the AHS where the relationship between exposure to glyphosate based herbicide and the risk of NHL and other cancer types have been studied. These are all case-control studies from various regions: Sweden (Hardell and Eriksson, 1999; Hardell *et al.*, 2002; Eriksson *et al.*, 2008), Australia (Fritschi *et al.*, 2005), Canada (McDuffie *et al.*, 2001; Pahwa *et al.*, 2012; Kachuri *et al.*, 2013), Midwestern United States (Iowa and Minnesota, Kansas, Nebraska, by De Roos *et al.*, 2003 (analysing Cantor, 1992; Hoar, 1986; Zahm, 1990), and France (Orsi *et al.*, 2009). The Australian study does not report on glyphosate itself ("other herbicides - mainly glyphosate and carbamates") and is not discussed further. A European multi-center lymphoma case-control study (Cocco *et al.*, 2013) was performed in 6 European countries (ES, FR, DE, IE, IT, CZ).

The case-control studies have a retrospective design, which introduces the possibility of recall bias among the participants that can influence the observed risk estimates. Proxy respondents are often used for subjects that have died or become incapacitated, adding further possibilities for bias and misclassification of exposure. RAC notes that as the use of pesticides is typically seasonal and occasional and often involves several pesticides, the retrospective assessment of such exposures, having occurred years or decades earlier, is prone to inaccuracies due to the participants recollection of use of glyphosate based herbicides, use of other pesticides, exposure duration and use of personal protective equipment.

¹ In the nested case-control study, cases of a disease that occur in a defined cohort are identified and, for each, a specified number of matched controls is selected from among those in the cohort who have not developed the disease by the time of disease occurrence in the case.



Statistical associations

Statistical null associations – solid tumours, leukemia and Hodgkin's lymphoma

No association was found between exposure to glyphosate based herbicide and the risk of solid tumours, leukemia and Hodgkin's lymphoma (HL) (De Roos *et al.*, 2005; Engel *et al.*, 2005; Flower *et al.* (2004), Koutros *et al.*, 2011; Lee *et al.*, 2004; 2005; 2007; Andreotti *et al.*, 2009; Band *et al.*, 2011; Pahwa *et al.*, 2011). No association between exposure to glyphosate based herbicide and increased risk of leukemia has been found; this was recently supported by Chang and Delzell (2016) in a meta-analysis of De Roos *et al.* (2005); Brown *et al.* (1990); and Kaufman *et al.* (2009). Chang and Delzell also investigated the risk of HL based on the studies by Karunanayake *et al.* (2012) and Orsi *et al.* (2009), and found statistically null associations with HL.

In relation to other cancer types, Mink *et al.* (2012) reviewed the quality of the following 7 cohort studies (nested case-control studies) all based on the AHS cohort: Flower (2004, childhood cancer), De Roos (2005, multiple cancer endpoints), Alavanja (2003, prostate cancer), Engel (2005, breast cancer), Lee (2007, colorectal cancer), Andreotti (2009, pancreatic cancer) and Dennis (2010, cutaneous melanoma). Mink *et al.* (2012) stated that all of the studies were prone to bias, measurement error, and/or confounding factors, and concluded that with a cautious interpretation of the few positive associations reported in the literature, the epidemiological data considered together do not support a causal association between glyphosate exposure and cancer. No meta-analysis was performed as the authors did not consider it appropriate to calculate quantitative summary relative risk estimates across studies evaluating different site-specific cancers.

RAC agrees with the DS that there is no epidemiological evidence of an association between exposure to glyphosate based herbicide and the risk of solid tumours, leukemia or HL among the studies presented in the CLH report.

In the public consultation, a study reporting a positive association between exposure to pesticides and risk of cutaneous melanoma was submitted. This study is discussed separately below.

Statistical associations – NHL and MM

No association between exposure to glyphosate based herbicide and the risk of NHL was found in the AHS, where 92 cases of NHL were observed during a median follow-up time of 6.7 years (De Roos *et al.*, 2005), with a rate ratio (RR) of 1.1, 95 % confidence interval (CI) 0.7–1.9 adjusted for age, demographic and life-style factors and exposure to other pesticides. Glyphosate exposure was not associated with NHL incidence overall or with any of the cancer subtypes studied. No dose-response relationship was observed between NHL incidences and cumulative exposure days or intensity-weighted exposure days of glyphosate use. There was, however, a *suggested association* with MM incidence that the authors recommended to be followed up as more cases occur in the AHS, with reported a RR of 2.6 (95% CI 0.7-9.4) (the most fully adjusted, De Roos *et al.* 2005).

Statistically significant associations between exposure to glyphosate based herbicide and NHL have been reported in case-control studies in the Swedish, Canadian and U.S. populations. However when adjustment for confounding factors was applied, the effects were no longer statistically significant in most studies. In the Swedish case-control study which included 910 cases of NHL and 1016 controls living in Sweden, 29 persons with NHL and 18 control persons

reported exposure to glyphosate giving an initial odds ratio¹ (OR) 2.02/CI 1.10-3.71 (Eriksson *et al.*, 2008), when adjusted for age, sex and year of diagnosis (cases) or enrolment (controls). When it was adjusted for co-exposure to other agents than glyphosate using multivariate analysis the adjusted OR was not statistically significant (OR 1.51, CI = 0.77-2.94). Hardell *et al.* (2002) found a significant increase of NHL in a Swedish case-control study which included 515 cases and 1141 controls (8 exposed cases and 8 exposed controls) when using univariate analysis with OR 3.04, CI=1.08-8.52, but it also became non-significant when applying a multivariate analysis (OR 1.85, 95% CI=0.55-6.20). Adjustments were made for use of other pesticides in the multivariate analysis. In Canadian men, McDuffie *et al.* (2001) reported an adjusted OR for NHL of 1.20 (95% CI 0.83-1.74), adjusted for age, province and medical variables (but not use of other pesticides) in a case-control study including 517 cases and 1506 controls. The OR was significant for only cases with more than 2 days exposure per year, compared to those with less (OR 2.12, CI=1.20-3.73.) In mid-western U.S. the risk for NHL when exposed to glyphosate was found to be statistically significantly increased with 36 exposed cases of NHL and 61 controls with logistic regression OR 2.1 (95 % CL 1.1-4.0) (De Roos *et al.*, 2003). Adjustments were made for use of other pesticides. When hierarchical regression was applied, the association was not statistically significant, with OR 1.6 (0.9 to 2.8). This was based on analyses of pooled data from three case-control studies (Cantor *et al.* 1992; Zahm *et al.*, 1990; Hoar *et al.*, 1986) from the NCI, including 622 cases/1245 controls, 201 cases/725 controls and 170 cases/948 controls, respectively. In analyses of multiple pesticides, there were 650 cases and 1933 controls following exclusion of subjects with missing data. In a French case-control study which included 244 cases and 436 controls, Orsi *et al.* (2009) did not find an increased risk (OR 1.0, 95% CI=0.5-2.2, of 12 exposed cases and 24 exposed controls).

Proxy respondents were used in the pooled analysis of three case-control studies by De Roos *et al.* (2003), and in the case-control studies by Hardell *et al.* (2002) and McDuffie *et al.*, 2001. Proxy respondents were not used by Eriksson *et al.* (2008) and Orsi *et al.* (2009).

In the hospital based case-control study reported by Orsi *et al.* (2009), face-to-face interviews were conducted with the patients. All the other case-control studies described here were population-based, and self-administered questionnaires were distributed to cases and controls. The self-administered questionnaires were followed up by telephone interviews for clarification in the studies by Eriksson *et al.* (2008), Hardell *et al.* (202), and McDuffie *et al.* (2001). The use of proxy respondents in some studies and questionnaire-based exposure information with the previously mentioned mentioned recollection related inaccuracy, both regarding exposure to glyphosate based herbicides and exposure to other pesticides, indicate that effects of confounding and bias cannot be ruled out in those studies or in the meta risk estimates relying on those studies. This is the case even if efforts were made to minimise them.

Exposure-response trend was investigated by De Roos *et al.* (2003) as multiple pesticide use, and by Eriksson *et al.* (2008) as exposure on more or less than 10 days per year, and by McDuffie as days/year of exposure (mixing or applying pesticides). It needs to be mentioned that RAC considers multiple pesticide use not to be representative of an exposure-response analysis with regard to glyphosate exposure. RAC notes that while some indication of a dose-response relationship was observed in the Eriksson *et al.* (2008) and McDuffie *et al.* (2001) studies, these analyses did not adjust for confounding by exposure to other pesticides.

¹ An odds ratio (OR) is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. Odds ratios are most commonly used to measure an association in case-control studies.

Odds ratios above 1 have been found in some case-control studies of MM, but without statistical significance (Brown *et al.*, 1993, 173 cases and 650 controls; Pahwa *et al.*, 2012, 515 cases and 506 controls). Re-analyses of the same cohorts have come to the same result.

Confounders and other obstacles to causal inference were described by the DS, such as:

- exposure to other constituents in glyphosate based herbicide
- exposure to other pesticides,
- use of questionnaires and interviews and
- poor recollection of exposure to glyphosate based herbicide,
- no measurement of blood biomarkers,
- lack of power due to small number of cancer cases,
- changes over time in the definition of NHL.

RAC notes that 'confounding' in epidemiology refers to a situation where a factor other than the one assessed correlates both with exposure and outcome, e.g. a co-formulant in glyphosate based formulations would be a confounder if it would be at the same time a risk factor for the outcome in question (cancer or more specifically NHL). Further, RAC notes that measured blood biomarkers would more securely indicate any correlation between exposure and NHL and that there are some biomonitoring data available, e.g. Curwin *et al.* (2007)¹. In this study, urinary levels of glyphosate were not higher among children, mothers, and fathers living in a farm household compared to families in non-farm households in Iowa, U.S. In fact, the glyphosate levels were higher among the non-farm children than the farm children. Covariates such as amount of pesticide applied, or playing in treated fields did not correlate with urinary levels. Niemann *et al.* (2015)² reported on 7 biomonitoring studies, also indicating low levels of glyphosate in human urine from both operators and consumers. RAC notes that the co-formulant Polyethoxylated (POE)-tallowamine (CAS No 61791-26-2) was until quite recently allowed to be used in glyphosate based herbicides in Europe. Since August 2016, 'Member States shall ensure that plant protection products containing glyphosate do not contain the co-formulant POE-tallowamine' (see Commission Implementing Regulation (EU) 2016/1313). According to the EFSA evaluation (2015), significant toxicity of POE-tallowamine has been observed for the endpoints for which data exists. However, no data are available regarding long-term toxicity and carcinogenicity of POE-tallowamine.

RAC acknowledges that due to their nature, epidemiological studies are subject to a greater level of uncertainty compared to experimental studies, since exposure and other conditions are not controlled by the investigator. Consequently, bias, confounding factors, inaccuracies in exposure assessment *etc.* need to be minimized when designing and performing an epidemiology study. RAC notes that epidemiology is a highly relevant way to study effects in humans, as is also acknowledged by the CLP regulation and guidance.

Reviews, re-analyses and meta-analysis of NHL and MM

Reviews and re-assessments of the AHS data were conducted by: Sorahan (2015), Alavanja *et al.* (2013), Mink *et al.* (2012) and Weichenthal *et al.* (2010). The Sorahan paper was not included in the CLH report, but was mentioned in the public consultation by a MSCA.

In a study sponsored by Monsanto, Sorahan (2015) re-analysed the data for MM reported by De Roos *et al.* (2005), and concluded that the risk given by De Roos (RR 2.6, 95% CI 0.7-9.4) was

¹ Mentioned in comment no. 161 in the public consultation.

² Mentioned by the DS in a reply to comment no. 126 in the public consultation.

due to an unrepresentative restricted dataset and that there was no convincing link between the glyphosate use and the risk of MM. When using the full dataset and adjusting for a) age and gender, and b) lifestyle factors, the RR decreased to 1.12 (95% CI 0.50-2.49) and 1.24 (95% CI 0.52-2.94), respectively.

Alavanja *et al.* (2013) did not re-analyse data but compiled results from multiple epidemiological studies of the relationship between exposure to pesticides and the risk of cancer. They mentioned one positive study by Eriksson *et al.* (2008) and the association between glyphosate and NHL, but other negative studies are not mentioned.

Mink *et al.* (2012) reviewed the quality 14 case-control studies to evaluate whether exposure to glyphosate was associated causally with risk of any type of cancer in humans. The case-control studies reporting on the relationship between exposure to glyphosate and risk of NHL were: Cantor (1992), Nordstrom (1998), Hardell and Eriksson (1999), McDuffie (2001), Hardell (2002), De Roos (2003), Lee (2004a), Eriksson (2008). Mink *et al.* (2012) stated that all of the studies were prone to bias, measurement error, and/or confounding, and concluded that with a cautious interpretation of the few positive associations reported in the literature, the epidemiological data considered together do not support a causal association between glyphosate exposure and cancer. No meta-analysis was performed as the authors did not consider it appropriate to calculate quantitative summary relative risk estimates across studies evaluating different site-specific cancers.

In a review of cancer incidence in 28 epidemiological studies of pesticide exposure and cancer incidence in the AHS cohort, Weichenthal *et al.* (2010) stated that glyphosate was not associated with NHL or any other cancer type in pesticide applicators. Exposure misclassification was mentioned as a concern.

In a meta-analysis the risk estimates (OR or RR) from several studies are combined in a way that the statistical accuracy of the study (size of the study) and not the magnitude of the risk estimate defines their weight in the overall weighted meta-RR. Still the meta-analyses carry over any potential bias or confounding that might be in the risk estimates of those individual studies, e.g. any effect that may come from recall bias or use of proxy respondents.

Systematic review and meta-analysis by Chang and Delzell (2016)

Chang and Delzell recently (2016) published a systematic review and meta-analysis, sponsored by Monsanto, on glyphosate exposure and risk of lymphohaematopoietic cancers. In the meta-analysis [i.a. on the following studies reporting on NHL and NHL subtypes: (De Roos *et al.*, 2005 and 2003; Eriksson *et al.*, 2008; Hardell *et al.*, 2002; McDuffie *et al.*, 2001; Orsi *et al.*, 2009; Cocco, 2013], they concluded that they found marginally significant positive meta-relative risks (meta-RRs) for the association between glyphosate use and risk of NHL (meta-RRs 1.3, 95% CI 1.0-1.6) when using the most adjusted risk estimate from the studies. In a meta-analysis of the studies of Orsi *et al.* (2009), Sorahan (2015), Brown (1993), and Kachuri (2013) there was a slight significant positive meta-RR for the association between glyphosate use and risk of MM (meta-RR 1.4, 95% CI 1.0-1.9). There were statistically null associations with HL based on the studies of Orsi (2009) and Karunanayake (2012) (meta-RR 1.1, 95% CI 0.7-1.6) and leukemia based on the studies of De Roos (2005), Brown (1990), and Kaufman (2009) (meta-RR 1.0, 95% CI 0.6-1.5). Even though there was a slight positive association between glyphosate use and NHL and MM, the authors could not substantiate a causal relationship due to considerations in light of the Bradford Hill causality criteria. The results are presented in the figure below, reproduced from Figure 1 in Chang and Delzell (2016). The authors selected the newer studies while still covering all available data from older publications.

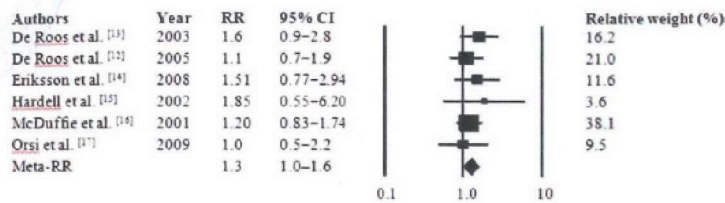


Figure 1. Forest plots of relative risk (RR) estimates and 95% confidence intervals (CIs) for the association between glyphosate exposure and risk of non-Hodgkin lymphoma. Meta-RRs were identical in random-effects and fixed-effects models.

Figure from Chang and Delzell, 2016

Chang and Delzell (2016) also analysed MM, and came up with the following forest plots:

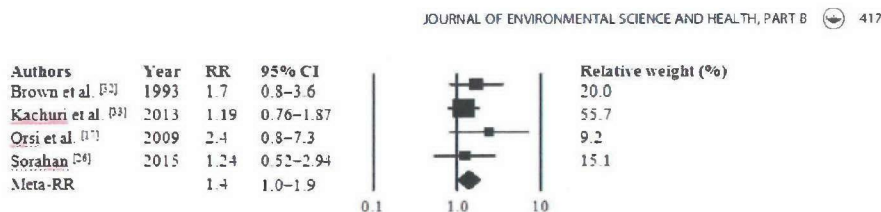


Figure 2. Forest plots of relative risk (RR) estimates and 95% confidence intervals (CIs) for the association between glyphosate exposure and risk of multiple myeloma. Meta-RRs were identical in random-effects and fixed-effects models.

Figure from Chang and Delzell, 2016

Systematic review and meta-analysis by Schinasi and Leon (2014)

A systematic review and meta-analysis for all studied populations was performed by the IARC scientists Schinasi and Leon (2014), who found a positive association between glyphosate use and NHL risk when the following studies were meta-analysed: McDuffie *et al.* (2001), Hardell *et al.* (2002), De Roos *et al.* (2003), De Roos *et al.* (2005), Eriksson *et al.* (2008), Orsi *et al.* (2009). The meta-risk ratio estimate for glyphosate and NHL was 1.5, 95% CI 1.1-2.0, and it was stronger (meta-RR 2.3, 95% CI=1.4-4.0) in the studies diagnosed in the period 1975-1989 compared to more recent periods. The strongest meta-RR estimates were associated with subtypes of NHL. For B cell lymphoma the meta-RR was 2.0 (CI 1.1-3.6) based on only two studies (Cocco, 2013 and Eriksson *et al.*, 2008), and identical to the result of Chang and Delzell (2016) based on the same studies. A possible causal relationship was not discussed by Schinasi and Leon (2014).

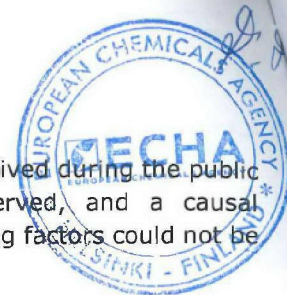
The IARC monograph working group addressed the same studies as Schinasi and Leon (2014), but used the most fully adjusted risk estimates from the articles by Hardell *et al.*, 2002, and Eriksson *et al.*, 2008. The resulting meta-RR for glyphosate and NHL was 1.3 (95% CI 1.03-1.65), i.e. the same as the meta-RR calculated by Chang and Delzell (2016, meta-RR 1.3, 95% CI 1.0-1.6), based on the same studies.

The Epilymph study of B-cell lymphoma was a part of the meta-analyses of both Chang and Delzell (2016), and Schinasi and Leon (2014), who both concluded on a meta-risk ratio estimate of 2.0, 95% CI 1.1-3.6, when the Epilymph study and Eriksson *et al.* (2008) were analysed.

IARC and EFSA

In 2015, IARC classified glyphosate as "probably carcinogenic to humans" (Group 2A), primarily based on animal studies. In their evaluation, the human data on carcinogenicity (primarily NHL)

was described as limited. In Portier *et al.* (2015 online, 2016 in print, received during the public consultation) it was explained that a positive association was observed, and a causal interpretation was considered credible, but that chance, bias or confounding factors could not be ruled out.



US EPA Report of the cancer assessment review committee (CARC, 2015)

This and several other recent review reports were mentioned in public consultation comment no. 216 (Monsanto/GTF). CARC concludes that the epidemiological evidence does not support a causal relationship between glyphosate exposure and solid tumours. Also for several types of non-solid tumours like HL and MM, CARC states that there is no evidence to support a causal relationship. However, for NHL, they say that evidence from epidemiology is inconclusive for a causal associative relationship with glyphosate exposure.

Other cancer types

Very few associations were found in the studies between glyphosate based herbicide and cancer types other than for NHL. Since publication of the dossier, a study with a pooled analysis of two case-control studies, which presented evidence of an association between exposure to pesticides and cutaneous melanoma (CM), was published (see Fortes *et al.*, 2016), and was mentioned in public consultation comment no. 185. The studies included 304 CM cases and 305 controls in Italy and 95 CM cases and 96 controls in Brazil. Every use of any pesticide was associated with a high risk of CM (odds ratio 2.58; 95% confidence interval 1.18-5.65) in particular exposure to herbicides (glyphosate reported as most used) and fungicides (mancozeb and maneb reported as most used), after controlling for confounding factors such as sex, age, skin photo-type and sun-burn episodes in childhood. It was reported that glyphosate was the most used of the herbicides. However, no separate statistical analyses were reported for glyphosate exposure and when the groups of pesticides were analysed, confounding for exposure to other types of pesticides was not controlled. There was a greater risk for cutaneous melanoma (OR 4.68; 95% CI: 1.29 to 17.0) for persons exposed to both pesticides and occupational sun exposure than for persons not exposed to sun during work.

Available epidemiological case-control studies, reviews, re-analyses and meta-analyses show weak statistically significant associations between exposure to glyphosate based herbicide and findings of cancer, especially NHL. This indicates a potential concern for human health. However, chance, bias and confounding factors could not be ruled out. A causal relationship with exposure to glyphosate based herbicide can thus not be confirmed by RAC. More specifically, this is due to a number of factors – i.a. the weak associations which were only significant when certain statistical tests were applied, small studies with low number of exposed cases, the probability of recall bias for previous exposure (duration and dose) especially in the case-control studies, the lack of biomonitoring data, frequently not adjusting for confounding factors such as co-exposure to other pesticides and risk estimates often getting lower when more comprehensive adjustment was applied, the presence of a toxic co-formulant (POE-tallowamine), and the changes in the definitions of NHL/other cancers over the years.

No association between exposure to glyphosate and incidences of NHL was observed in the only cohort study available.

The findings from the epidemiology studies are used in a weight-of-evidence approach together with the findings in animal studies. The comparison with the classification criteria is given in the next section.



Comparison with the CLP criteria

The database for the evaluation of glyphosate carcinogenicity is extensive and RAC bases their assessment on data from human epidemiological studies and a wide range of experimental animal carcinogenicity studies (7 rat and 5 mouse conventional cancer bioassays). The exposure route was oral in both the rat and the mouse studies and the doses used were sufficiently high in all but one of the evaluated studies. There are no data suggesting that there are significant species differences and the studies performed and the tumour types evaluated are considered relevant to humans. **The database includes studies of sufficient reliability and relevance to allow a robust evaluation following the requirements of CLP.**

Category 1A

Classification in category 1A concerns substances known to have carcinogenic potential for humans and is largely based on human evidence.

Although available epidemiological case-control studies, reviews, re-analyses and meta-analyses show weak statistically significant associations between exposure to glyphosate based herbicide and findings of cancer, especially NHL, chance, bias and confounding factors could not be ruled out. A causal relationship to cancer following exposure to glyphosate based herbicide can thus not be confirmed by RAC.

Hence, classification of glyphosate in category Carc.1A is not justified. The detailed reasoning has been provided above.

Category 1B

Category 1B is for substances presumed to have carcinogenic potential for humans. Classification is largely based on animal evidence.

Following an overall evaluation of the human evidence and the tumour data from 7 rat and 5 mouse bioassays it is concluded that there is not sufficient evidence for carcinogenicity and a classification of glyphosate in category 1B is thus not warranted. The evaluation of strength of evidence and additional considerations including biological relevance of the tumour data is provided for each tumour type above. The main arguments are briefly summarised below.

Category 2

Category 2 substances are suspected human carcinogens. Classification is based on evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. RAC notes the following in relation to glyphosate:

Epidemiological data:

- No association between exposure to glyphosate and cancer was found in the AHS, which is the only prospective cohort study available. A weak positive association has been observed in some case-control studies, and in meta-analyses between exposure to glyphosate and cancer, especially NHL, as concluded in the meta-analyses by Chang and Delzell (2016) and Schinasi and Leon (2014), and also in IARC monograph 112. A causal relationship could not be established by RAC because chance, bias, and confounding factors could not be ruled out, and the evidence from epidemiological studies was considered insufficient to demonstrate carcinogenicity in humans. The increased risk observed in some case-control studies was not consistently observed in all case-control studies nor in the only cohort study available. When the whole database of epidemiology is taken into consideration, RAC concludes that the criteria for assigning glyphosate to category 2 (or any of the other categories) are not fulfilled.

Animal bioassays:

- There is insufficient evidence to support a classification in category 2 based on the evaluation of seven rat studies. A significant increase in benign pancreatic tumours, was observed in males in the low dose groups of two studies (Lankas, 1981; Stout and Ruecker, 1990), but no apparent dose-response relationships were seen. No similar increase in tumour incidences was reported for female rats in these two studies and no similar indication of pancreatic tumours were observed in any of the five other long-term studies for either males or females. The same holds true for liver adenomas and thyroid C-cell adenomas that were increased only in the study by Stout and Ruecker (1990). The incidences of liver adenomas were within, whereas the incidences of thyroid tumours were slightly above, the range of the historical controls. The conclusion is supported by the benign nature of the tumours with no suggestions of progression towards malignancy, a low strength of the evidence and a lack of consistency between sexes and across the many studies performed.
- In the mouse, three tumour types were considered in detail. These were renal tubular tumours, haemangiosarcomas and malignant lymphomas. An increase in renal tumours was reported in males in the high exposure group in three of the five studies. Increase incidences in haemangiosarcoma was reported in CD-1 males at the top dose in two studies, and an increased incidence of malignant lymphoma was reported in three carcinogenicity studies in CD-1 mice and one study in Swiss albino mice. The increases in tumour incidences were all non-significant in pairwise comparisons with control groups by the Fisher's exact test. However, several of the findings were significant when tested by the Cochran-Armitage trend test. RAC considered that the findings in the individual mouse studies were not by themselves strong enough to warrant classification. This is based mainly on an evaluation of statistical significance, biological relevance and consistency of the findings, including comparison with historical control data and differences in findings between the sexes. Increased tumour incidences observed at doses above 4000 mg/kg bw/day were given less weight by RAC because the doses used were excessive and exceeded the MTD. Looking at the overall pattern of tumour incidences, RAC notes a tendency for increased incidences of malignant lymphomas in male mice in the high dose groups in four of the five studies available. However, the tumour incidences were highly variable, mostly within the available control incidences, and elevated tumour incidences were not supported by parallel increases in non-neoplastic lymph node lesions. Furthermore, the findings were not consistent between sexes and were not supported by findings in the rat studies.
- Mode of action data: Glyphosate is not reactive and no structural similarity to a substance(s) for which there is good evidence of carcinogenicity has been suggested. RAC does not find sufficient evidence to support a genotoxic MoA for glyphosate. Furthermore, the available data do not support non-genotoxic modes of action such as growth stimulation or tissue necrosis. Immunosuppression is a recognised risk factor for NHL, but the data for glyphosate is regarded as insufficient for evaluation of this endpoint.

RAC concludes that based on the epidemiological data as well as the data from long-term studies in rats and mice, taking a weight of evidence approach, no classification for carcinogenicity is warranted.

J. J.



RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The DS noted that the reproductive toxicity potential of glyphosate was investigated in a large number of two-generation studies in rats, only 6 of which could be considered either fully valid or supplementary. These studies were summarised in Table 46 of the CLH report, along with (what the DS described as) a "deficient" three-generation study.

The DS noted the existence of three additional reproductive toxicity studies which had been referred to in an earlier EU evaluation (Germany, 1998). No adverse effects were reported in any of these studies, but the DS did not consider them to be suitable for the purpose of classification and labelling. In the three-generation studies by Schroeder and Hogan (1981) and by Bhide (1988a,b), the top dose levels were considered much too low to reveal any toxic effect. A further published reproductive toxicity study (Dallegrave *et al.*, 2007) was performed using a commercial formulation and thus was also not considered useful for assessing classification and labelling of the active substance.

According to the DS, effects on the offspring were indicated by a reduced pup weight or weight gain in most studies but were confined to very high, parentally toxic dose levels. Furthermore, the relevance of the epidemiological data for detecting effects of glyphosate on fertility or reproductive performance was considered limited. Therefore, no classification for sexual function and fertility was considered warranted.

Development

The CLH report summarised a large number of developmental toxicity and teratogenicity studies with glyphosate conducted in rats and rabbits.

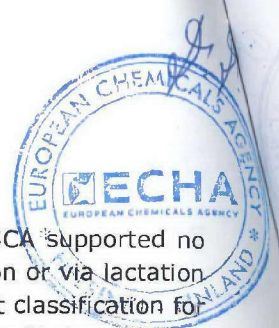
The studies did not show any teratogenic potential in rats. At 3500 mg/kg bw/d, which resulted in maternal toxicity and in one study even mortality, post-implantation loss and both skeletal variations and retardations were observed (Brooker *et al.*, 1991; Tasker and Rodwell, 1980). In the most recent study by Moxon (1996), no effects were seen at up to 1000 mg/kg bw/d, i.e., the highest dose tested.

In another study, no effects were seen in dams or in foetuses when the test substance was administered up to a daily dose of more than 500 mg/kg bw/d (approx. 10000 ppm) via the diet (Anonymous author, but the DS stated that the author could be Antal, 1981).

Overall, the rat studies revealed only slight developmental effects, which were confined to very high and maternally toxic dose levels.

In rabbits, developmental effects (which included dilated heart, visceral malformations and ventricular septal defects as well as retarded ossification or supernumerary rib in some studies) and, in addition, post-implantation loss were observed. The DS attributed these findings to glyphosate administration to the female rabbits. However, the DS also noted that these findings were confined to dose levels at which severe maternal toxicity was apparent.

The DS therefore concluded that based on animal studies no classification for developmental toxicity was warranted. Furthermore, the DS noted that no convincing evidence of reproductive or developmental effects of glyphosate could be derived from epidemiological studies or from *in vitro* or *in vivo* studies relevant to reproductive toxicity assessment.



Comments received during public consultation

A number of comments received during PC addressed this endpoint. One MSCA supported no classification for reproductive toxicity, but noted that a conclusion on effects on or via lactation was not included in the CLH proposal. Two MSCAs and 1 individual argued that classification for developmental toxicity could be relevant. One MSCA emphasized some of the effects observed in the reported studies as well as inconsistencies in the documents submitted for PC. They also provided references to other published data which was not included in the CLH report. This MSCA suggested classification as Repr. 2. One government authority (not an MSCA) concluded that glyphosate should be classified at least as Repr. 2, H361.

One comment from an individual referred to a publication describing concern for birth defects. Other comments from individuals or on behalf of an organisation supported classification as (at least) Repr. 2; H361, some explicitly supporting classification as Repr. 1B. One comment on behalf of an organisation indicated concerns for endocrine disruptive effects and low dose effects on reproduction. A further two organisations and one individual commented on the epidemiological studies and potential associations between glyphosate containing herbicides and miscarriage and ADHD.

One comment from an Industry organisation supported no classification. Another organisation commented on the low-dose effects and absence of a dose-response relationship. One of these comments referred to effects on male reproductive organs.

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

There are a large number of two-generation studies in rats available for glyphosate. The DS took six of these into account for the purpose of classification (table below: modified from Table 46 from the CLH report). In addition, one three-generation study with rats (Antal, 1985), was included in the evaluation by the DS, although the DS considered this study to have major reporting deficiencies and as such to present supplementary data only. The study did not show any treatment related effects at doses up to 5000 ppm (462-502 mg/kg bw/d).

Reproductive (two-generation) studies with glyphosate in rats (based on Table 46 from the CLH report)

Study, purity of glyphosate	Strain, route	Dose levels	NOAEL	LOAEL	Targets/ Main effects***
Dhinsa <i>et al.</i> , 2007; 95.7%	Sprague-Dawley, diet	0, 1500, 5000, 15000 ppm (corresponding to approximately 0, 105, 351 and 1053 mg/kg bw/d)	Parental, offspring, reproductive: 5000 ppm (351 mg/kg bw/d)	Parental, offspring, reproductive: 15000 ppm (1000-1600 mg/kg bw/d)	Parental: liver, kidney wt↑ in females; Repro: homogenisation resistant spermatid count↓ (399.9 million/g in controls vs 309.0 million/g at 15000 ppm in F0); Off-spring: delay in preputial separation in F1 males; day 45.9 vs 43 days in control. Not associated with reduced bw. No effects on fertility in F1 generation.
Moxon, 2000;	Wistar-	0, 1000,	Parental,	Parental,	Parental, offspring:



Study, purity of glyphosate	Strain, route	Dose levels	NOAEL	LOAEL	Targets / Main effects***
97.6%	derived AlpK, diet	3000, 10000 ppm (corresponding to approximately 0, 100, 293 and 985 mg/kg bw/d)	offspring: 3000 ppm (293 mg/kg bw/d); Reproductive: 10000 ppm (985 mg/kg bw/d)	offspring: 10000 ppm (985 mg/kg bw/d); Reproductive: not established	bw↓ (F1 pups & F1-adults)
Takahashi, 1997; 94.61%	Sprague-Dawley, diet	0, 1200, 6000, 30000 ppm (corresponding to approximately 0, 83, 417 and > 2000 mg/kg bw/d)	Parental, offspring: 6000 ppm (417 mg/kg bw/d); Reproductive: 30000 ppm (>2000 mg/kg bw/d)	Parental, offspring: 30000 ppm (>2000 mg/kg bw/d); Reproductive: not established	Parental: loose stool, bw↓, caecum distention, organ wt changes; Offspring: bw↓, caecum distention
Suresh, 1993*; 96.8%	Wistar rat, diet	0, 10, 100, 1000, 10000 ppm (corresponding to approximately 0, 0.8, 8, 80 and 800 mg/kg bw/d)	Parental, offspring, reproductive: 10000 ppm (800 mg/kg bw/d)	-	No treatment related effects
Brooker <i>et al.</i> , 1992**; 99.2%;	Sprague-Dawley, diet	0, 1000, 3000, 10000 ppm (corresponding to approximately 0, 66, 197 and 668 mg/kg bw/d)	Parental, offspring: 3000 ppm (197 mg/kg bw/d); reproductive: 10000 ppm (668 mg/kg bw/d)	Parental, offspring: 10000 ppm (668 mg/kg bw/d); Reproductive: not established	Parental, offspring: bw↓, food & water ↑, cellular alterations of salivary glands in F0/F1 m/f
Reyna, 1990; 97.67%;	Sprague-Dawley rat, diet	0, 2000, 10000, 30000 ppm (corresponding to approximately 0, 152, 760 and 2280 mg/kg bw/d)	Parental, offspring, reproductive: 10000 ppm (720-760 mg/kg bw/d)	Parental, offspring, reproductive: 30000 ppm (~2000 mg/kg bw/d)	Parental: bw gain↓, soft stool; Reproductive: litter size ↓ (equivocal); Offspring: bw gain↓

*supplementary study since dose levels might have been too low and no effects were seen at all

**supplementary range-finding one generation study (Brooker *et al.*, 1991) also available but without impact on classification and labelling

*** "main effects" were statistically significant if body weight and organ weights or reproductive parameters (apart from reduced litter size in the study by Reyna, 1990) were affected.

RAC examined each of these studies and found most of them to be acceptable for the assessment of classification. However, the study by Suresh (1993) was marked as a supplementary study since a LOAEL could not be derived. The study by Brooker *et al.* (1992) a range-finding one-generation study was regarded as supplementary.

The study by **Dhinsa *et al.* (2007)** was considered as acceptable. In this study a reduction in homogenisation resistant spermatid count (399.9 million/gram in controls vs 309.0 million/gram at 15000 ppm ~1000 mg/kg bw/d) was seen in the F0 generation. However, this was not reported in the F1 generation. A significant delay in sexual maturation, seen as delayed preputial separation in F1 male pups, was also observed at dose levels of 15000 ppm. Preputial separation

occurred after 45.9 days on average, compared to 43 days in the control group. However, this was not considered to be related to changes in F1 male bodyweight since the body weight was statistically significantly increased in the males with delayed preputial separation (body weight in controls 210g compared to 230g at 15000 ppm). The delayed onset of sexual maturation had no impact on subsequent reproductive performance. There were no treatment related effects on mating performance, fertility and gestation length in F0 and F1 generations. Further, no differences in litter size and viability was seen. The only systemic toxicity reported was a statistically significant increase in female liver and kidney weight (absolute and relative) in the high dose group in the F0 generation and in the liver weight (absolute and relative) in the F0 generations. During public consultation, a study by Dai *et al.* (2016) was also assessed, investigating effects of glyphosate on reproductive organs in male rats. The dose levels of glyphosate used were 0, 5, 50 and 500 mg/kg bw/d for 5 weeks with 8 rats/group. The only effects reported were a dose-dependent statistically significant reduction in seminal vesicle gland and coagulating gland weights (0.42, 0.37, 0.34, and 0.31 g in the 0, 5, 50 and 500 mg/kg bw/d dose group, respectively). Total sperm count was reduced in the high dose group, but without any clear dose-response relationship. No statistically significant changes were reported in the serum levels of testosterone, estradiol or progesterone. In the other two-generation studies, no significant effects were reported on sperm quality or male reproductive organs at doses up to 2000 mg/kg bw/d.

The **Moxon (2000)** study was considered as acceptable. In this study doses up to 970 mg/kg bw/d did not reveal any effects on mating performance, fertility, gestation and litter size in the F0 and F1 generations. Sperm assessment did not reveal any effects in either generation. No effects on pup body weight was reported at birth in the F1 and F2 generations. However, in male offspring from postnatal day (PND) 8 to 29 a statistically significant decrease in body weight was reported and in female offspring from PND 5 to 29 in the high dose group. In the F2 offspring no changes in body weight were reported. No effects on sexual maturation were reported in F1 males and females.

The **Takahashi (1997)** study was considered as acceptable. In this study, doses up to 2000 mg/kg bw/d did not reveal any effects on mating performance, fertility and litter size in F0 and F1 generations. The gestation index (%) was reduced, but not statistically significantly (95.8, 95.8, 87.5 and 79.2% in the control, 83, 417 and > 2000 mg/kg bw/d dose groups, respectively). Sperm assessment did not reveal any effects in any of the generations. General toxicity was reported in the F1 and F2 generations as loose stool and caecum distension in males and females and a decrease in male body weight in the high dose group. In the F1 and F2 offspring a statistically significant decrease in body weight from PND 14 and a significant increase in caecum distension was reported in the high dose group. Effects on sexual maturation were not assessed in this study.

The study by **Reyna (1990)** (not included in RAR and no information provided regarding acceptability) showed a rather equivocal reduction in litter size at dose levels exceeding 2000 mg/kg bw/d. In the two litters produced by the F0 generation, a non-significant reduction of litter size by up to 10 % was observed. This effect was less pronounced in the F1 generation. A reduction in litter size was not confirmed in the study by Takahashi (1997), where the same dietary concentrations of glyphosate were tested.

Human data

Several epidemiological studies investigating a possible impact of glyphosate exposure on fertility are available. The parameters included in the studies are fecundity, miscarriage, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects and the occurrence of attention-deficit disorder / attention-deficit hyperactive disorder

AD/ADHD) in children. However, a statistically significant positive association for these findings considered to be lacking.



Comparison with the CLP criteria

Repr. 1A

There are no clear indications of effects on fertility following exposure of glyphosate to humans, therefore RAC considers that a classification of glyphosate with Repr. 1A is not justified.

Repr. 1B

According to the CLP criteria, classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on reproductive toxicity in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

Repr. 2

According to the CLP criteria, classification of a substance in Category 2 is justified when there is some evidence from humans or experimental animals, possibly supplemented with other information of an adverse effect on sexual function and fertility, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be a more appropriate classification.

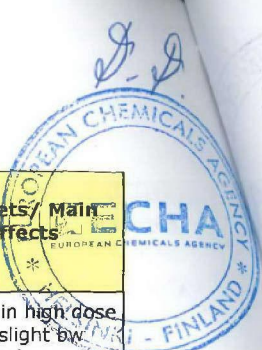
RAC concludes that the six two-generation reproductive toxicity studies and the study by Dai *et al.* (2016) did not provide any evidence of effects of glyphosate exposure on fertility or on the male and female reproductive organs. Further, no effects on sexual maturation in males and females was reported in the studies where this parameter was assessed. The effects seen were of equivocal relevance and were confined to high dose levels (>1000 mg/kg bw/d) and were seen in the presence of parental toxicity. Classification as Repr. 1B or Repr. 2 is hence not considered justified.

Effects on development

The DS included six developmental toxicity studies in rats and seven studies in rabbits in their evaluation of developmental toxicity following exposure to glyphosate. It should be noted that RAC also assessed the original full study reports (Robust Study Summaries are included in the RAR, Annex 7). The studies in **rats** are summarised in table below:

Developmental toxicity studies in rats (from the CLH report)

Study, purity of glyphosate (study quality)	Strain, route, duration of treatment	Dose levels	NOAEL	LOAEL	Targets/ Main effects
Moxon, 1996; 95.6% (acceptable in RAR)	Alpk (Wistar derived), gavage, GD 7-16	0, 250, 500, 1000 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Not applicable	None
Hatakenaka, 1995 95.68% (acceptable in RAR)	CD (SD), gavage, GD 6-15	0, 30, 300, 1000 mg/kg bw/d	Maternal & developmental: 300 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Maternal: Loose stool Development: skeletal anomalies seen in all doses but not considered treatment related
Brooker <i>et al.</i> , 1991,	CD, gavage,	0, 300,	Maternal &	Maternal &	Maternal: two



Study, purity of glyphosate (study quality)	Strain, route, duration of treatment	Dose levels	NOAEL	LOAEL	Targets/ Main effects
98.6% (acceptable or at least supplementary in RAR)	GD 6-15	1000, 3500 mg/kg bw/d	developmental: 300 mg/kg bw/d	developmental: 1000 mg/kg bw/d	deaths in high dose group, slight bw gain↓, noisy respiration and gaseous distension in GI tract (2/25) ; Development: ossification↓, skeletal anomalies at low incidences
Suresh, 1991,; 96.8% (supplementary in RAR)	20 x Wistar, gavage, 30 x controls, GD 6-15 pre-GLP	0, 1000 mg/kg bw/d	Maternal: 1000 mg/kg bw/d; Developmental: <1000 mg/kg bw/d	Maternal: not applicable; Developmental: 1000 mg/kg bw/d	Maternal: no effects; Development: ossification↓
Tasker and Rodwell, 1980; 98.7% (acceptable or at least supplementary in RAR)	Charles River, gavage, GD 6-19	0, 300, 1000, 3500 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Maternal & developmental. 3500 mg/kg bw/d	Maternal: mortality, soft stool, diarrhoea; Development: bw↓, post-implantation loss
Anonymous (author could be Antal), 1981; purity 96.8% (acceptable or at least supplementary in RAR)	CFY, diet, GD 6-18	Calculated to be 0, 22, 103, 544 mg/kg bw/d	Maternal & developmental: 544 mg/kg bw/d	Not applicable	None

Four of the six studies reported no evidence of developmental toxicity in rats. Only two of the studies reported results that required an in-depth analysis of the data by RAC (Tasker and Rodwell, 1980 and Brooker *et al.*, 1991).

The study by **Tasker and Rodwell (1980)**, tested doses up to 3500 mg/kg bw/d. At this very high dose, excessive maternal toxicity was reported including mortality (6/25 dams died). Up to the limit dose of 1000 mg/kg bw/d only weak maternal effects such as gastrointestinal signs including soft stool and diarrhoea or a lower bodyweight gain were seen. Post-implantation loss was observed; 4.2, 1.4, 3.1 and 14.3% in the 0, 300, 100 and 3500 mg/kg bw/d dose groups, respectively. The foetal body weight was statistically significantly reduced at 3500 mg/kg bw/d (3.5, 3.7, 3.6 and 3.2 g at 0, 100, 300 and 3500 mg/kg bw/d, respectively). The number of malformed foetuses were as follows: 3 in 3 litters, 0, 0 and 10 in 3 litters at 0, 100, 300 and 3500 mg/kg bw/d. In the high dose group, the malformations included six foetuses from one litter with a syndrome of bent tail, open eyelids, missing kidneys and ureters as well as various skeletal effects. Three foetuses in another litter were reported to have dwarfism. All the malformations were reported to be within the historical control data range. RAC concludes that the effects reported (post-implantation loss and malformations, the latter was reported to be within the range of the historical control data) were seen at a very high dose levels (3500 mg/kg bw/d) that caused excessive maternal toxicity (~25% of the dams died during the study). According to the CLP criteria (Annex I: 3.7.2.4.4) data from a dose level with such an excessive toxicity should normally not be considered for further evaluation.

In the study by **Brooker et al. (1991)**, maternal toxicity was evident at the high dose level as two mortalities and signs of salivation post-dosing, wet coats, noisy respiration/gasping and loose

faeces as well as gaseous distention of the GI tract. A marked reduction in body weight gain during the first two days of treatment and a slight reduction in body weight gain during GD 12-14 was also reported together with a reduced food intake during the dosing period. In the mid-dose group, noisy respiration was reported in 2/25 dams together with a slight reduction in bw gain during the 2 first days of dosing. A total of 23, 23, 25 and 22 dams had live pups at GD 20 in the control, 300, 1000 and 3500 mg/kg bw/d dose groups, respectively. There were no abortions and no total resorptions. Implantation rate, post-implantation loss and litter size were similar in all groups. Evidence of delayed ossification, increased incidence of foetuses with wavy ribs and reduced foetal weight was recorded at 1000 mg/kg bw/d (Table below). RAC considers that the effects on fetal weight and on the degree of ossification are secondary effects, due to the maternal toxicity observed in the high dose group and notes that an increase in wavy ribs was not recorded in any of the other available developmental toxicity studies. A total of 1 foetus from 1 litter, 2 from 2 litters, 1 from 1 litter, 9 and 3 from 2 litters in the control, 300, 1000 and 3500 mg/kg bw/d dose groups, respectively, were malformed (**foetal incidence: 0.3, 0.8, 0.3 and 1.1%, respectively**). The malformations observed were as follows: In the control group there was one foetus with markedly distended urinary bladder. In the 300 mg/kg bw/d group there was one small foetus (2.24 g vs approximately 4 g in control group) with left microphthalmia and one foetus with termination of vertebral column at the 1st sacral vertebra. These two foetuses were from different litters. In the 1000 mg/kg bw/d group one foetus had an interventricular septal defect and absent innominate artery. In the 3500 mg/kg bw/d group there was one small foetus (1.53 g) with an interventricular septal defect, palatine irregularity, nasopharyngeal fistula and subcutaneous oedema and atelectatic lungs; one foetus with palatine irregularity with misshapen basisphenoid and connected 5th to 6th right cervical vertebral arches; and one foetus with cervical irregularities, including one absent right, shortened 1st left and reduced ossification of cervical vertebral arches. RAC notes that a minimal increase in the foetal incidence of malformations was reported in the high dose group (see above). However, these were not statistically significant and showed no dose-response relationship for the single incidences of ventricular septal defect in the mid- and high dose groups. RAC therefore concludes that no evidence of developmental toxicity was reported in this study.

Foetal effects attributable to treatment in rats (Brooker et al., 1991)

Dose level (mg/kg bw/d)	0	300	1000	3500
Mean foetal wt (g)	3.96	3.90	3.89	3.71**
Foetuses with wavy ribs (thoracic ribs) / number of foetuses examined	1/155	-/143	3/166	28/144
Reduced ossification of 1 or more cranial centres	3/155	2/143	12/166	10/144
Reduced ossification of sacrocaudal vertebral arches	3/155	8/143	17/166	15/144
Foetuses with unossified sternbrae (%)	13.7	28.5	17.6	33.8**
Foetuses showing skeletal variation (%) ¹	11.7	22.6	28.4	35.7**

* statistically significant, p < 0.05; ** p < 0.01

¹Historical control range for skeletal variations: 21.9 – 27.2%

Hatakenaka (1995) showed a slight increase in skeletal variations including lumbar ribs (11 foetuses from 7 litters compared to 4 foetuses from 2 litters in control animals) at doses of 1000 mg/kg bw/d. External malformations included a short tail in one foetus of the 30 mg/kg bw/d group and microphthalmia in one foetus of the 1000 mg/kg bw/d group. Visceral examination

revealed ventricular septal defects in one foetus of each of the 300 and 1000 mg/kg bw/d groups and another foetus (from a different litter) at 300 mg/kg bw/d displayed a right aortic arch. Skeletal malformations were rare and were not associated with treatment, the incidences being similar in all groups (2, 0, 2 and 3 fetuses had malformations in the control group, 30, 300 and 1000 mg/kg bw/d groups, respectively). The malformations included splitting of ossification centers of the thoracic vertebral bodies and asymmetry of the sternebrae with sternocostal joint displacement. During the dosing period in the 1000 mg/kg bw/d group, 20 out of 22 pregnant females showed slightly loose stool and the increase in its incidence was statistically significant. There were no mortalities. Maternal toxicity was considered as minimal. RAC concludes that no evidence of developmental toxicity was reported in this study.

Suresh (1991) performed this study as a supplementary limit test in Wistar rats with only two groups; a control group and a 1000 mg/kg bw/d group. Mortality and clinical signs of toxicity were not evident. The incidence of foetal malformations was not increased relative to controls. A significantly increased incidence of delayed ossification (normal variations) including caudal vertebral arch, forelimb proximal phalange and hindlimb distal phalanges were reported at 1000 mg/kg bw/d. RAC concludes that this limit test did not result in any increased incidences of external, visceral or skeletal malformations.

The most recent study by **Moxon et al. (1996)** showed no effects at doses up to 1000 mg/kg bw/d. One control animal was killed on day 7 as a result of being misdosed. There was no evidence of maternal toxicity or effects on the foetuses. The incidence of foetuses with major defects was 1/284, 1/297, 1/301 and 2/296 in the control and 250, 500 and 1000 mg/kg bw/d groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate. The defects were dissimilar in type and of single incidence. Further, the proportion of foetuses with external/visceral variants and the proportion of foetuses with skeletal variants were lower in the glyphosate treated groups than in the control group. RAC concludes that no evidence of developmental toxicity attributable to glyphosate was reported in this study.

Summary of rat developmental toxicity studies

In one of the the six studies in rats (Tasker and Rodwell, 1980) effects were observed (post-implantation loss and malformations, the latter reportedly within the historical control data range) at a very high dose level (3500 mg/kg bw/d) that caused excessive maternal toxicity (~25% of the dams died during the study). According to the CLP legislation (Annex I: 3.7.2.4.4) data from a dose level with such an excessive toxicity should normally not be considered for further evaluation. RAC concludes that no classification for development is justified according to the CLP criteria based on this study.

Cardiovascular malformations were reported in two of the six studies with rats. In the study by Hatakenaka *et al.* (1995) it was reported as single incidences at 300 and 1000 mg/kg bw/d, and were not considered related to maternal toxicity. In the study by Brooker *et al.* (1991), single incidences of cardiovascular malformations were reported at 1000 and 3500 mg/kg bw/d in the presence of maternal toxicity only at 3500 mg/kg bw/d. RAC concludes that due to the single incidences of cardiovascular malformations without a clear dose-response relationship and without statistical significance in the six rat developmental toxicity studies, no classification for development is justified according to the CLP criteria based on the studies in rats.

In the table below, the the main effects seen in the seven developmental toxicity studies in rabbits following exposure to glyphosate are summarised. Further information on maternal

toxicity is included in the STOT RE section in the table "Rabbit maternal mortality and toxicity from developmental studies with glyphosate".



Developmental toxicity studies in rabbits¹ (from the CLH report)

Study, purity of glyphosate (study quality)	Strain, duration of treatment, route	Dose levels	NOAEL	LOAEL	Targets/ Main effects
Coles and Doleman, 1996; 95.3%. GLP (study acceptable in RAR)	NZW rabbit, GD 7-19, gavage. 18 rabbits/dose group	0, 50, 200, 400 mg/kg bw/d	Maternal & developmental: 50 mg/kg bw/d	Maternal & developmental: 200 mg/kg bw/d	Maternal effects at the high dose: diarrhoea and scours, mortality (2 deaths), stat. sign. ↓ bw gain and food consumption; Development: stat. sign. ↑ post-implantation loss at mid dose
Moxon, 1996; 95.6%. GLP (study acceptable in RAR)	NZW rabbit, GD 8-20, gavage. 20 rabbits/dose group	0, 100, 175, 300 mg/kg bw/d	Maternal: 100 mg/kg bw/d; Developmental: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d; Developmental: 300 mg/kg bw/d	Maternal: in high dose group ↓ food intake and stat. sign. bw gain ↓, diarrhoea; Development: foetal wt stat. sign. ↓ in high dose group, ossification retarded. Minor skeletal defects
Hojo, 1995, 97.56%. GLP (study acceptable in RAR)	Japanese White rabbits (Kbl:JW), GD 6-18, gavage. 18 rabbits/dose group	0, 10, 100, 300 mg/kg bw/d	Maternal: 100 mg/kg bw/d; Developmental: 300 mg/kg bw/d	Maternal: 300 mg/kg bw/d; Developmental: not applicable	Maternal: mortality (1 death), loose stool, abortions (2 in low and high dose group). No effects on food intake or bw; Development: stat. sign. ↑ in % of litters with skeletal malformations at 300 mg/kg bw/d.
Suresh <i>et al.</i> , 1993*; 96.8%. GLP (study supplementary in RAR)	NZW rabbit, GD 6-18, gavage. 26, 17, 16 and 15 rabbits in the 0, 20, 100 and 500 mg/kg bw/d dose groups	0, 20, 100, 500 mg/kg bw/d	Maternal: 20 mg/kg bw/d; Developmental: 100 mg/kg bw/d	Maternal: 100 mg/kg bw/d; Developmental: not established due to low number of foetuses at top dose	Maternal: mortality (4 deaths at mid and 8 at high dose), soft/liquid stool; stat. sign. ↓ food consumption and bw and bw gain in high dose. Development: no clear-cut effects up to 100 mg/kg bw/d (in high dose group low number of foetuses and litters, but stat. sign. increase in visceral malformations in all dose groups (dilated heart)
Brooker <i>et al.</i> , 1991; 98.6%. GLP (study acceptable in RAR)	NZW rabbit, GD 7-19, gavage. 19, 19, 16 and 20 rabbits in the 0, 50, 150 and 450 mg/kg	0, 50, 150, 450 mg/kg bw/d	Maternal: 50 mg/kg bw/d; Developmental: 150 mg/kg bw/d	Maternal: 150 mg/kg bw/d; Developmental: 450 mg/kg bw/d	Maternal: mortality following abortion (1 at top dose), clinical signs (GI-tract), food intake and bw gain ↓; Development: late embryonic death,



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Study, purity of glyphosate (study quality)	Strain, duration of treatment, route	Dose levels	NOAEL	LOAEL	Targets/ Main effects
	bw/d dose groups				post-implantation loss, cardiac malformations
Bhide & Patil, 1989**; Lot 38, 95% Study has serious deficiencies. Not GLP (study supplementary in RAR)	NZW rabbit, GD 6-18. Gavage. 15 rabbits/dose group	0, 125, 250, 500 mg/kg bw/d	Maternal & developmental: 250 mg/kg bw/d	Maternal & developmental: 500 mg/kg bw/d	Maternal effects in high dose: food intake stat. sign. ↓ and bw↓, 2 abortions; Development: malformations (external, visceral & skeletal)
Tasker <i>et al.</i> , 1980*; 98.7%. Adhere to GLP (study supplementary in RAR)	Dutch Belted rabbit, GD 6-27, gavage. 16, 16 and 16 rabbits in the 0, 75, 175 and 350 mg/kg bw/d dose group	0, 75, 175, 350 mg/kg bw/d	Maternal: 75 mg/kg bw/d; Developmental: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d; Developmental: not established due to low number of foetuses	Maternal: mortality (1, 2 and 10 at low, mid and high dose), soft stool, diarrhoea. No effects on maternal bw and bw gain; Development: none up to 175 mg/kg bw/d (high dose group excluded and not assessed. Due to maternal mortality only 6 litters were available at c-section.

* supplementary study since high dose group could not be evaluated for developmental toxicity/teratogenicity

** study with serious deficiencies in conduct and reporting

¹Detailed study summaries are included in the Annex 7 of the "Renewal assessment Report" (p 620 – 669)

The developmental toxicity studies showed that pregnant rabbits are more sensitive than pregnant rats to the exposure to glyphosate.

Severe maternal toxicity seen as treatment-related premature deaths, were reported in several studies at doses ranging from 100 to 500 mg/kg bw/d. Many of the female rabbits that died or were killed *in extremis* seem to have severe effects in the GI tract including ulceration. A possible explanation for the greater sensitivity of pregnant rabbits compared to pregnant rats following exposure to glyphosate may be because rabbits ingest their caecotrophes (a specialized digestive strategy for the recycling of caecal contents and the extraction of nutrients). This may lead to two outcomes in the rabbits:

- 1) Glyphosate as well as other substances that predominantly are excreted unchanged in the faeces, can be readily available for repeated oral uptake and the caecotroph may therefore constitute a potential source of increased exposure to glyphosate in rabbits relative to other species, including humans. This possible recycling of glyphosate and increased exposure in rabbits might explain the particular sensitivity of this species;
- 2) Maternal toxicity was reported as soft stools and diarrhoea and these effects may prevent the rabbits from ingesting their caecotrophs, and consequently the overall well-being of the rabbits would be affected. Further information regarding the pre-mature deaths is included in the table "Rabbit maternal mortality and toxicity from developmental studies with glyphosate" in the STOT RE section.

According to the CLP Regulation, maternal mortality greater than 10 % is considered excessive and the data from this dose level shall not normally be considered further for evaluation (CLP

Annex 1: 3.7.2.4.4). However, following exposure to glyphosate some of the premature deaths was reported to be related to treatment with glyphosate while others were due to mis-gavage or infections.

In the section below, the two studies requiring in-depth analysis for effects on foetal viability are summarised followed by the six studies requiring in depth analysis for foetal pathological findings.

Effects on foetal viability

An overview of the observed foetal pathological effects is presented in Table A in the section "Supplemented information – in depth analysis by RAC".

Effects on embryo-foetal viability, which can be revealed by analyzing a number of parameters (e.g. viable litter size at C-section, post-implantation loss, number of early and late embryo-foetal death and number of dead foetuses) that are interlinked in one way or another to each other, were only reported in two of the available studies, i.e. in Coles and Doleman (1986); and in the study by Brooker *et al* (1991) (see Table A in the section "Supplemental information – in depth analysis by RAC" for an overview of the observed effects on fetal viability in the available rabbit developmental toxicity studies).

In the study by **Coles and Doleman (1996)** (described as acceptable in the RAR) performed with NZW rabbits, a slightly increased number of post-implantation loss was recorded at the two highest dose levels. However, the dose-response relationship in the increase in post-implantation losses was not considered to be convincingly (mean % of post-implantation loss: 3.7 ± 6.5 , 3.6 ± 8.5 , 11.5 ± 11.4 and 12.1 ± 18.6 in the 0, 50, 200 and 400 mg/kg bw/d dose groups respectively). In the high dose group (400 mg/kg bw/d) the slight, but not statistically significant increase in late embryo/foetal deaths and post-implantation loss was considered not to be related to treatment, since it was mainly due to one animal that had nine late embryonic/foetal deaths (resulting in a post-implantation loss of 69.2% in that specific animal). In addition, the mean viable litter size at C-section was similar at all dose levels (9.1 ± 2.5 , 8.7 ± 2.4 , 7.9 ± 2.5 and 8.9 ± 2.6 in the control, low, intermediate and high dose group, respectively) and consequently the slight, but statistically significant, increase in post-implantation loss (mainly caused by a non-statistically significant increase in early embryonic/foetal death) that was observed at the intermediate dose level is considered to have limited biological relevance. Further, no dose-related or statistically significant effect was recorded on foetal weights at any dose levels up to and including 400 mg/kg bw/d (41.5 ± 5.5 , 39.4 ± 5.6 g, 41.7 ± 4.5 and 38.2 ± 5.2 in the control, low, intermediate and high dose groups, respectively). At the highest dose level, maternal toxicity was observed as a statistically significant decrease in body weight gain from GD 10-29 with clinical signs that included diarrhoea and scours, as well as premature death of two female rabbits (one died at GD 19 and one was killed in extremis on GD 20). The macroscopic necropsy findings of the 2 female rabbits included fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, congested duodenum and gas distended colon, rectum and appendix. In the intermediate dose (200 mg/kg bw/d), maternal toxicity was evident as a decrease in bw gain, however, it was not statistically significant. At this dose level one female was found dead on GD 16 and necropsy findings in the lungs indicated that the death was due to technical complications during dosing. At the low dose, no mortality occurred. In the control group, one doe was found dead two minutes after dosing and necropsy findings in the lungs indicated mal-dosing. Overall RAC concludes that the increase in post-implantation loss was of low biological relevance.

In the study by **Brooker (1991)** (considered acceptable in the RAR) a similar degree of increase in post-implantation loss was recorded at all dose levels (19.5 ± 19.8 , 15.3 ± 17.2 and $21 \pm$

11.8 at 50, 150 and 450 mg/kg bw/d, respectively), compared to controls (5.7 ± 7.2), see table below. Although a dose-related decrease of the mean litter size at C-section was noted, the reduction in the litter size was small and not statistically significant. RAC notes the absence of a dose-response relationship for the post-implantation loss and that according to the available historical control data (based on 21 studies performed during 1989 and 1990; range: 6.5 – 17.5; median 12.9) there was a great variability in post-implantation loss in rabbits in the test facility where this study was performed. Maternal toxicity was reported as one maternal death at the top dose of 450 mg/kg bw/d on GD 20 following abortion, gastrointestinal disturbances, reduced food intake and pronounced body weight loss (- 660g) as well as few haemorrhagic depressions in the stomach. Female rabbits that survived in the two highest dose groups showed reduced food consumption compared to the controls, but these were not statistically significant. In the mid dose at 150 mg/kg bw/d a reduction of 12% compared to controls was observed from GD 11-19. At 450 mg/kg bw/d this was also evident throughout the treatment period with reductions of 6-17 % during GD 7-19. No statistically significant effect on absolute maternal bw was recorded throughout the study, but a slight decrease in bw gain that coincided with the reduction in food consumption was recorded during GD 11-20 at the mid dose (-32% less than controls) and top dose (-46%), respectively (table B.6.6-43 in the RAR). A dose related increase in females showing soft/liquid faeces were seen at the two highest doses.

No similar effect on post-implantation loss were recorded in the studies by Moxon (1996) and Hojo (1995) where dose levels up to 300 was used, or in the study by Suresh *et al.* (1993) with dose-levels up to 500 mg/kg bw/d. In the study by Bhide and Patil (1989) where dose levels up to 500 mg/kg bw/d was used a slightly higher mean number of embryo/foetal death (1.4 ± 2.20 as compared to 0.07 ± 0.26 in the control) and a slightly lower mean number of viable implants/litter (5.2 ± 3.03 as compared to 7.3 ± 3.1 in the control) was reported. However, the study by Bhide and Patil (1989) had serious deficiencies in conduct and reporting, no statistical analysis was provided and since data from the 2 high-dose dams that aborted during the study was included in the analysis it is not clear to what extent this data influenced the outcome of the data analysis and consequently the data from this study should be handled with caution, and will not be taken into account in the overall weight of evidence analysis.

Summary of maternal and litter parameters (group mean values) in rabbits from the study by Brooker *et al.* (1991) from the CLH report

Parameter	Dose Group (mg/kg bw/d)				Historical control range (mean value)
	0 (Control)	50	150	450	
No. of mated females	19	19	16	20	--
No. not pregnant	0	6	1	5	--
No. of premature deaths	0	0	0	1 [§]	
No. of female rabbits with live pups or litters at day 29	18	12	15	13	--
Reduced faecal output	9	8	11	12	
Soft/liquid faeces	0	2	5	13	--
Corpora lutea	11.5	12.4	11.7	11.3	9.0 – 12.9 (11.2)
Implantations	9.7	10.5	9.0	9.2	7.0 – 11.1 (9.5)
Pre-implantation loss	14.6	15.4	23.4	18.8	2.3 – 26.1 (15.1)
Early embryonic deaths	0.4	0.9	0.9	0.5	0.3 – 1.1 (0.6)
Late embryonic deaths	0.2	0.9	0.5	1.3**	0.1 – 1.3 (0.7)
Abortions	0.0	0.0	0.1	0.0 [‡]	0.0 – 0.1 (0)
Total embryonic deaths	0.6	1.8*	1.5*	1.8**	0.6 – 2.0 (1.2)

Parameter	Dose Group (mg/kg bw/d)				Historical control range (mean value)
	0 (Control)	50	150	450	
Post-implantation loss (%)	5.7	19.5*	15.3*	21.0**	6.5 – 17.5 (12.9)***
Live pups	9.1	8.7	7.5	7.3	6.1 – 9.5 (8.3)
Litter weight (g)	389.5	370.6	320.5	315.0	281.9 – 402.2 (352.9)
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--

- § Day 20, following abortion on the day before
 * Statistically significant by Kruskal –Wallis 'H' test P < 0.05
 ** Statistically significant by Kruskal –Wallis 'H' test P < 0.01
 *** Historical control data: 8.1% (2.8-17.7) Holson *et al.*, 2006 and 9.1% (0.6 – 23.4) (MARTA, 1997)
 # Fisher's exact test follow-up by intergroup comparison with control was not statistically significant p > 0.05

Overall RAC concludes that following *in utero* exposure to glyphosate in rabbits no clear relationship between exposure and effects on foetal viability could be determined. Effects on foetal viability were not reported consistently in the four acceptable developmental toxicity studies in rabbits. Actually, only one study (Brooker *et al.*, 1991) reported effects on foetal viability, however, without a clear dose-response relationship and within the historical control range for late- and total embryonic deaths.

Foetal pathological findings

An overview of the observed foetal pathological effects is presented in Table B in the section "Supplementary information – in depth analysis by RAC".

In five out of seven developmental toxicity studies performed in rabbits, foetal skeletal and visceral malformations were reported, but at low incidences and in the study where historical control data were available (Brooker *et al.*, 1991), they were within the range of the historical control data. The foetal skeletal and visceral malformations were also reported in the presence of severe maternal toxicity including death and GI tract intolerance. However, the deaths were reported to be both substance related and due to technical problems with the dosing of the animals or related to infections. An assessment of the five studies are included below.

In the study by **Moxon et al. (1996)** (described as acceptable in the RAR) performed with NZW rabbits, the number of foetuses (litters) with major defects were 3(2), 1,0 and 2(2) in the controls, low, intermediate and high dose groups, respectively. One foetus at the 100 and 300 mg/kg bw/d dose levels was reported to have a single heart ventricle, thickened ventricle walls, enlarged aorta and reduced pulmonary artery, whereas one control fetus was reported to have an enlarged aorta and a persistent truncus arteriosus. In the high dose group there was also one fetus with gross malformations of the skull. A statistically significant increase in foetuses (litter) with minor skeletal defects was reported in the low- and high dose group (58 (16), 82 (18), 59 (16) and 79 (17) at 0, 100, 175 and 300 mg/kg bw/d). However, when looking at the individual minor skeletal effects, a statistically significant increase was recorded only in the high dose group for the following observations: partially ossified transverse process on the 7th cervical vertebrae (8 foetuses in 2 litters as compared to 1 foetus in the controls), unossified transverse process on the 7th lumbar vertebrae (14 foetuses in 4 litters as compared to 4 foetuses in 3 litters in the controls) or partially ossified 6th sternebrae (16 foetuses from 7 litters as compared to 4 foetuses in 2 litters in the controls). It should also be noted that the foetal bw was statistically significantly reduced in the top-dose group (44.4g in controls and 40.7g at 300 mg/kg bw/d). A statistically significant increase in foetuses (litter) with skeletal variations was also reported in the high dose

group (119 (17), 129 (18), 116 (17) and 132 (17) at 0, 100, 175 and 300 mg/kg bw/d). These variations included an increase (but not statistically significant) in the incidence of fetuses with partially ossified odontoids (62 fetuses in 15 litters as compared to 50 fetuses in 15 litters in the controls) or 27 pre-sacral vertebrae (37 fetuses in 12 litters as compared to 23 fetuses in 10 litters in the controls). Abortions occurred in 1, 2, 1 and 2 rabbits in the 0, 100, 175 and 300 mg/kg bw/d dose groups. All animals that aborted died or were sacrificed *in extremis*. In the high dose group, a statistically significant reduction in maternal body weight gain was reported and was accompanied by a reduction in food consumption. RAC concludes that the minor and major defects did not show a clear dose-response with increasing dose, and were also reported in the control group, and therefore not considered related to treatment.

As revealed by Table B (see Supplementary information section, and in Table B6.6 – 52 in Annex 7 to the RAR), the main finding at the external visceral and skeletal examination in the study by **Suresh et al. (1993)**, considered to be supplementary in the RAR, was cardiovascular malformations (summarised in the table below). This study using NZW rabbits, showed that the percentage of fetuses with "dilated heart" was significantly increased at all dose levels. At 20 mg/kg bw/d, 4 cases of dilated heart were reported with 2 cases in one litter and 1 case in each of 2 litters. At 100 mg/kg bw/d, 3 cases of dilated heart was reported in 1 litter and 1 case in another litter, and at 500 mg/kg bw/d 4 cases of dilated heart was reported in one litter and 1 case in another litter. No definition of the recorded dilated heart or information regarding the historical control data for dilated heart was included by the DS or in the study report. Foetal weight were statistically significantly increased in the low and mid-dose groups (32, 35, 35, 33 g in the 0, 20, 100, 500 mg/kg bw/d dose groups, respectively). There were no significant maternal effects in the doe with 3 cases of dilated heart at 100 mg/kg bw/d. In the doe with 4 cases of dilated heart at 500 mg/kg bw/d, soft stool and diarrhoea was recorded at GD 10. Further information regarding maternal toxicity included that 4/16 females in the mid dose and 5/15 females in the high dose group died during the dosing period (Table below). In addition 3 females in the high dose died after cessation of substance administration. It is noted that in the control group two females also died, however, this was considered to be due to mis-dosing during gavage. Some uncertainties are also described relating to the cause of the premature death in the 100 and 500 mg/kg bw/d dose groups since various findings in the lungs and trachea, suggestive of gavage errors, were recorded at gross necropsy in 5/8 (high dose) and in 1/4 (intermediate dose) female rabbits that died before the end of the study. These findings may indicate that the premature death may be related to gavage errors but the unclear findings following necropsy in some of these animals makes this inconclusive. RAC concludes that the high incidence of maternal deaths is considered to lead to an insufficient number of fetuses being available for assessment from the high dose group (i.e 28 fetuses from 5 litters). Further, RAC considers that the reporting of cardiovascular malformations was insufficient due to a lack of measurements of the heart and that no definition of the diagnosis was provided in the study report. No information regarding the historical control data for dilated heart was included by the DS or provided in the study report.



Summary of mortality in female rabbits in the study by Suresh *et al.* (1993)

Parameter	Dose Group (mg/kg bw/d)			
	0 (control)	20	100	500
Mated females	26	17	16	15
Dead during treatment	1*	0	4	5
Died post-treatment	1*	0	0	3
Total number of deaths	2	0	4***	8**
% mortality	7.7	0.0	25.0	53.3

*Animal died due to mis-gavage

**5 out of 8 female rabbits had lung lesions (emphysema, collapsed, pneumonic lesions, consolidated and congested)

***1 out of 4 female rabbits that died had lung and trachea congestion and froth in trachea

Cardiovascular malformations in the rabbit study of Suresh *et al.*, (1993)

Dose group (mg/kg bw/d)	0	20	100	500
No. of foetuses/no. of litters examined	133/20	78/13	77/12	28/5
Major visceral malformations:				
No. of foetuses/litters with dilated heart	-	4*/3	4*/2	5*/2*
No. of foetuses/litters with cardiomegaly	0	0	1 ^A	0
No. of foetuses/litters with "seal shaped" hearts	1/1	0	1 ^A	0
No. of foetuses/litters with dilated ventricle	1/1	0	1/1	1/1
No. affected/total no. of foetuses	2/133	4/78	4/77	5/28
Litters affected/total no. of litters	2/133	3/13	2/12	2/5

* statistically significant, $p \leq 0.05$

^A same fetus

In the study by **Brooker *et al.* (1991)** (described as acceptable in the RAR) performed with NZW rabbits, the number of foetuses (litters) with major malformations were 3(3), 3(2), 5(3) and 6(5) in the control, low, intermediate and high dose groups. Single incidences (usually only found at one dose level) of some major malformations were identified in the cranial, lumbar or lumbar/sacral region of the foetus. Malrotated hindlimbs/forelimb flexure and/or hindlimb/forelimb brachydactyly were also reported with a foetal (litter) incidence of: 0, 2(2), 1(1) and 1(1) at the control, low, intermediate and high dose levels, respectively.

However, the main finding in the study by Brooker *et al.* (1991) was the recording of different cardiovascular malformations (see table below). Interventricular septal defects were recorded at the highest dose, and were seen in 4 foetuses from 4 litters (i.e. at an incidence outside the historical control data). The same effects were seen in one foetus from each of the other dose groups, including the control group. Other cardiovascular malformations of low incidence (but still outside the historical control data) were; enlarged left ventricles, reduced right ventricles, retro-oesophageal right subclavian artery and narrow/dilated aortic arch/pulmonary trunk/arterial trunk. It should however, be noted that in the high dose group interventricular septal defect, enlarged left, reduced right ventricles and narrow/dilated aortic arch/pulmonary trunk/arterial trunk originated from two foetuses from two different litters. Retro-oesophageal



right subclavian artery was reported in two foetuses from the same litter, one of these foetuses were also reported to have interventricular septal defect. Thus, the cardiovascular malformations were to some extent clustered together in the same foetuses. In the mid-dose group all three foetuses with retro-oesophageal right subclavian artery were from the same litter (see table below). Maternal toxicity was reported as one maternal death at the top dose of 450 mg/kg bw/d on GD 20 following abortion, GI disturbances, reduced food intake and body weight loss. Females in the two highest dose groups showed reduced food consumption compared to the controls, but these were not statistically significant. In the mid-dose at 150 mg/kg bw/d a reduction of 12% was observed from GD 11-19. At 450 mg/kg bw/d this was also evident throughout the treatment period with reductions of 6-17 % during GD 7-19. No changes in maternal bw throughout gestation were reported. A dose related increase in females showing soft/liquid faeces and signs of lack of appetite were seen at the two highest doses. However, in the top dose group there was no clear correlation between the severity of the maternal toxicity and the fetuses with interventricular septal defects. RAC concludes that the reported increase in cardiovascular malformations were to some extent clustered together in the same fetuses and was shown in the presence of maternal toxicity, however, it was not considered marked.

Summary of foetal parameters in rabbits in the study by Brooker *et al.* (1991) (From the CLH report, with some modifications)

Parameter	Dose Group (mg/kg bw/d)				Historical control range or x/y \bar{x} (mean)
	0 (control)	50	150	450	
Number of female rabbits with live pups or litters at Day 29	18	12	15	13	--
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 - 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--
Malformations					--
Total number of foetuses examined	163	104	112	95	1511
Number of malformed foetuses (%)	3 (1.9)	3 (5.8)	5 (4.3)	6 (5.9 (F))	51 (0.7 - 5.9 (3.8))
Number of affected litters (%)	3 (16.67)	3 (25)	3 (20)	5 (38.5)	43/188 (22.9)
Cardiovascular malformations					--
Number of foetuses with interventricular septal defect (%)	1 ^K (0.6)	1 ^J (1.0)	1 ^F (0.9)	4 ^{A,B,C,D} (4.2)	10/1511 (0.66)
Litter incidence (%)	1 (5.6)	1 (8.3)	1 (6.7)	4 (30.8)	10/188 (5.3)
Foetuses with enlarged left, reduced right ventricles (%)	0 (0)	0 (0)	0 (0)	2 ^{B,D} (2.1)	2/1511 (0.13)
Litter incidence (%)	0 (0)	0 (0)	0 (0)	2 (15.4)	2/188 (1.10)
Foetuses with retro-oesophageal right subclavian artery (%)*	0 (0)	0 (0)	3 ^{G,H,I} (2.7)	2 ^{A,E} (2.1)	7/1511 (0.46)
Litter incidence (%)	0 (0)	0 (0)	1 (6.6)	1 (7.6)	7/188 (3.72)
Foetuses with narrow/dilated aortic arch/pulmonary trunk/arterial trunk (%)	1 ^K (0.6)	1 ^J (1.0)	1 ^F (0.9)	3 ^{B,C,D} (3.2)	8/1511 (0.52)
Litter incidence (%)	1 (5.56)	1 (8.3)	1 (6.67)	3 (23.1)	8/188 (4.25)
Anomalies					--

Parameter	Dose Group (mg/kg bw/d)				Historical control range or x/y ^o (mean)
	0 (control)	50	150	450	
Total number of fetuses examined [#]	160	101	107	89	
Number of fetuses with gross/visceral anomalies (%)	9 (6.4)	14 (19.5)	14 (12.9)	6 (9.6 (K))	--
Number of fetuses with skeletal anomalies (%)	21 (11.7)	13 (17.7)	14 (12.5)	11 (10.1 (K))	--
Number of fetuses with reduced ossification (%)	7 (4.4)	4 (4.0)	5 (4.7)	4(4.5)	--
Mean foetal weight of fetuses with reduced ossification (g)	37.9	43.6	37.7	26.1	--

- ◊ Number affected / total number examined
- # Malformed fetuses are excluded
- * Retrooesophageal right subclavian artery is considered a variation by other laboratories (Solecki *et al.*, 2014)
- (F) Fisher's exact test applied, not statistically significant ($p > 0.05$)
- (K) Kruskal-Wallis 'H' statistic, not significant ($p > 0.05$)
- no data
- A,B,C,D,E,F,G, H, I, J, K - Represents different fetuses

The study by **Bhide and Patil (1989)** (regarded as supplementary in the RAR) performed with NZW rabbits was described to have several serious reporting deficiencies, including no individual data, no statistical analysis, no uterine weights and no results from maternal necropsy. Further, no historical control data was included in the study report. Maternal toxicity was reported in the high dose group as lower food consumption and reduced bw gain. In this study the total number of fetuses and litters with malformations were higher at 250 and 500 mg/kg bw/d relative to controls (3 fetuses (3 litters), 6(6), 10(10) and 20(14) from the 0, 125, 250 and 500 mg/kg bw/d dose groups, respectively) and included ventricular septal defects (0(0), 1(1), 1(1) and 2(2) fetuses (litters) from the 0, 125, 250 and 500 mg/kg bw/d dose groups, respectively). Other malformations included abnormal tail (foetal (litter) incidence of 1(1), 1(1), 2(2) and 2(2)), absent kidney(s) (foetal (litter) incidence of 1(1), 2(2), 2(2) and 6(6)), absent postcaval lung lobe (fetal (litter) incidence of (0, 1(1), 2(2) and 3(2)) and rudimentary 14th rib (fetal (litter) incidence of 1(1), 0, 2(2) and 5(2)). No information regarding statistical significance was included in the study. It is not clear from the reporting of the study whether the different malformations were found in different fetuses or if some fetuses had multiple malformations. The total number of litters in the high dose with malformations is reported to be 14. However, the number of animals on the study was 15 and out of these 3 were reported as being nonpregnant and 2 as having aborted. However, the number of litters examined is reported to be 12 in the high dose group which implies that aborted fetuses were examined and that data from these 2 litters were included in the analysis. RAC concludes that due to serious reporting deficiencies in the study the results from this study should be treated with great caution.

The developmental toxicity study by **Hojo (1995)** (acceptable in the RAR) was performed with Japanese white rabbits with doses of glyphosate at 0, 10, 100 and 300 mg/kg bw/d. In this study a statistically significant increase in the numbers of litters with skeletal malformations were reported. The litter/foetus incidences were 1/1 (5.6/0.7%), 3/4 (20/3.1%), 2/6 (12.5/4%) and 5/5 (35.7/4.5 %) in the 0, 10, 100 and 300 mg/kg bw/d dose groups, respectively. The most frequent malformations were fissure (0, 1, 3 and 0 fetuses in the low-, mid- and high-dose group, respectively) or splitting (0, 0, 3 and 1 fetuses in the low-, mid- and high-dose group, respectively) of the parietal bones. In the low- and high-dose groups, 1 foetus and 2 fetuses had fusion of parietal bones. The impact of the increase in skeletal malformations was difficult to interpret since a litter is counted whether only one or all fetuses are affected, and for most of

the skeletal malformations 1-2 fetuses/litter were affected. Visceral malformations were reported in one fetus at 10 mg/kg bw/d (fusion of the right pulmonary lobe and dilatation of the lateral ventricles). At 100 mg/kg bw/d, two fetuses from the same litter had fusion of the right pulmonary lobe and one of the fetuses also had undescended testis. One fetus from another litter had hypoplasia of the pulmonary arteria with ventricular septal defects. However, it is noted that no similar effect on the craniofacial skeleton was recorded in the other acceptable rabbit studies at dose levels up to and including 500 mg/kg bw/d. The maternal toxicity reported included one maternal death in the high dose group, abortions (2 in low and 2 in high dose group) and loose stool. No effects were reported on food intake or body weight. RAC concludes that the skeletal craniofacial malformations reported at low incidences in one study but not found in the other six rabbit developmental toxicity studies were considered to be anomalous and were given less weight in the overall weight of evidence.

The developmental toxicity study by **Tasker (1980)** (supplementary in RAR) was performed with Dutch belted rabbits with doses of glyphosate at 0, 75, 175 and 350 mg/kg bw/d. In this study the number of fetuses (litters) with malformations were 0, 3(3), 2(2) and 2(1) from the 0, 75, 175 and 375 mg/kg bw/d dose groups, respectively. Soft tissue malformations were reported in two fetuses in the high dose group (one with carpal flexure and one with gastro-thoraco-schisis and foetal anasarca). Skeletal malformations were reported in the low- and mid-dose groups (encephaly, absent rib, malformed rib and fused cervical vertebral centre). The maternal toxicity reported included maternal death (0, 1, 2 and 10 in the 0, 75, 175 and 350 mg/kg bw/d dose groups), soft stool and diarrhoea. No effects on maternal body weight and body weight gain was reported. RAC consider that the high incidence of maternal deaths (10 female rabbits died) in the high dose group leads to an insufficient number of litters being available for assessing possible adverse effects on foetal development at 375 mg/kg bw/d in this study.

In summary, the increases in interventricular septal defects in the study by Brooker *et al.* (1991), the increase in ventricular septal defects in the study by Bhide and Patil (1989) and the increase in the incidence of dilated heart in the study by Suresh (1993) may give some concern for the induction of visceral malformations in the heart following *in utero* exposure to glyphosate in rabbits. However, the studies by Bhide and Patil (1989) and Suresh (1993) were reported to have serious deficiencies. In the studies by Suresh (1993) and Tasker (1980) high maternal death was reported in the high dose group (500 mg/kg bw/d and 350 mg/kg bw/d) leading to insufficient number of fetuses being available for assessment. Furthermore, the cardiovascular malformation related to treatment with glyphosate was not reported consistently in the seven developmental toxicity studies in rabbits, and when reported the incidences were low and without clear dose-response relationship and were also reported in the control groups. An increase in cranial bone malformations (fissure and or splitting of parietal bones) was reported in the study by Hojo (1995). However, no similar finding was reported in the other acceptable studies in rabbits.

Human information

Several epidemiological studies investigating a possible impact of glyphosate exposure on development are available. However, there seems to be a lack of statistically significant positive associations and the concurrent exposure to glyphosate formulations and other chemicals makes it difficult to establish a positive link between exposure and effects when the results cannot be directly attributed to the pure active substance *per se*.

In two studies in which the subjects were in residential proximity to pesticide applications in California, no association was found between early gestational exposure to glyphosate

malformations were observed in the foetus from the fusion of the lobe and dilatation of the foetus from the... however, ... able

formulations and increased risk of hypospadias or neural tube defects and orofacial clefts in offspring (Carmichael *et al.*, 2013 and Yang *et al.*, 2013).

The incidence of spontaneous abortions was studied in Canada with pre-conception exposure to glyphosate (Arbuckle *et al.*, 2001). Out of 3936 pregnancies, 395 abortions were reported (10%) however, the baseline rate of spontaneous abortion in the general population was 12-15%. Recall bias of spontaneous abortion was also indicated in this study so no clear conclusion can be drawn.

It is expected that human *in utero* exposure to glyphosate would be nearly negligible, since the perfusion rate of glyphosate across the placenta is reported to be low. In the study by Mose *et al.*, (2008) the *ex vivo* transfer of glyphosate from maternal circulation to the foetal circulation was shown to be 15 %. In addition, the systemic intake of glyphosate is calculated to be low in the general population. In a study performed in 43 pregnant women in Australia the daily intake level was calculated to be 0.001 mg/kg bw/d (McQueen *et al.*, 2012). In comparison, the acceptable daily intake (ADI) for glyphosate in the EU is 0.5 mg/kg bw/d (EFSA, 2015).

In summary, there is no convincing evidence of developmental effects following *in utero* exposure to glyphosate from epidemiological studies.

Comparison with the CLP criteria

Repr. 1A

There are no clear indications of effects on development following exposure of glyphosate to humans, therefore RAC considers that classification of glyphosate as Repr. 1A is not justified.

Repr. 1B

According to the CLP criteria, classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on development is considered not to be a secondary non-specific consequence of other toxic effects and for Repr. 2;

Repr. 2

According to the CLP criteria, a classification of a substance in Category 2 is justified when there is some evidence from humans or experimental animal, possible supplemented with other information of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

In six developmental toxicity studies performed in rats, no consistent adverse effects were reported on development and RAC considers that classification for developmental toxicity is not justified based on these studies.

In the seven developmental toxicity studies performed in rabbits, some evidence of adverse effects on development were observed in five of the studies (all performed in different laboratories, three described as acceptable in the RAR) at dosage levels far lower than those used in the rat studies and thus indicating that pregnant rabbits are a more sensitive species than the pregnant rat following oral exposure to glyphosate. The developmental toxicity reported included statistically significant increases in late embryo-foetal death, post-implantation loss as well as skeletal and visceral malformations, although at low incidences, which for some of the effects was without a clear dose-response relationship and not consistently reported in all seven rabbit developmental toxicity studies. It should be noted that only 4 of the 7 studies were

considered to be acceptable in the RAR and by RAC. Two studies were supplementary in the RAR because a limited number of litters were available at the high dose group for evaluation of effects on embryofetal development, and one study had serious reporting deficiencies. RAC has taken the acceptability of the available studies into account in the overall weight of evidence analysis of the total data set.

Post-implantation loss and late/early embryo-foetal death was reported in only two (acceptable quality) out of the seven rabbit studies. Based on the weight of evidence RAC concludes that following *in utero* exposure to glyphosate in rabbits no clear relationship between exposure and effects on foetal viability could be determined. Effects on foetal viability were not reported consistently in the four acceptable developmental toxicity studies in rabbits. Only one study (Brooker et al., 1991) reported effects on foetal viability, however, without a clear dose-response relationship and within the historical control range for late- and total embryonic deaths.

Visceral and skeletal malformations were reported in five (three acceptable) out of the seven rabbit studies. Based on the weight of the evidence, RAC concludes that the reported increases in visceral malformations including interventricular septal defects in the study by Brooker *et al.* (1991), the increase in ventricular septal defects in the study by Bhide and Patil (1989) and the increase in dilated heart in the study by Suresh (1993) gives some evidence that cardiovascular malformations in the heart can be induced following *in utero* exposure to glyphosate in rabbits. The studies by Bhide and Patil (1989) and Suresh (1993) were reported to have serious deficiencies. In the study by Suresh (1993) and Tasker (1980) high maternal death was reported in the high dose group (500 mg/kg bw/d and 350 mg/kg bw/d) leading to insufficient number of foetuses being available for assessment. The cardiovascular malformations related to treatment to glyphosate was not reported consistently in the seven developmental toxicity studies in rabbits, and when reported, the incidences were low, without a clear dose-response relationship and were also reported in the control groups. As regards skeletal malformations, this was reported in the study by Hojo *et al.* (1995); however, a statistically significant increase in skeletal craniofacial malformations were not seen in the other acceptable rabbit developmental toxicity studies.

In conclusion, the six studies studies with rats with doses up to 3500 mg/kg bw/d showed insufficient evidence of developmental toxicity following *in utero* exposure to glyphosate including reduced ossification and skeletal malformations at maternally toxic doses, with a LOAEL for developmental effects ≥ 1000 mg/kg bw/d.

In the seven developmental toxicity studies in rabbits, limited evidence of cardiovascular malformations, skeletal malformations, post-implantation loss and embryo-foetal death were reported following *in utero* exposure to glyphosate since no clear picture of these effects were reported across the seven rabbit developmental toxicity studies. These effects were reported at low incidences, and in some of the studies without a clear dose-response relationship. Further, it should be noted that the cardiovascular malformations were to some extent clustered together in the same foetuses. Skeletal malformations evident as craniofacial malformations was reported in one study (Hojo, 1995), however, it is noted that no similar malformations were recorded in the other six acceptable studies at dose levels up to and including 500 mg/kg bw/d. The effects were reported in the presence of severe maternal toxicity including death of the female rabbits and GI tract intolerance to glyphosate exposure. However, it should be kept in mind that some of the deaths were related to mis-gavage and therefore not substance related. Furthermore, in some of the studies serious deficiencies in the reporting of the results were evident.

Epidemiological studies show no convincing evidence of developmental effects following *in utero* exposure to glyphosate.

Overall, RAC concludes that no classification for developmental toxicity is justified.

Elementary in the RAR
 evaluation of effects
 RAC has taken
 place safely
 EUROPEAN CHEMICALS AGENCY
 HELSINKI - FINLAND



ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to retain the classification as Aquatic Chronic 2 (H411).

Degradation

Glyphosate was hydrolytically stable at pH values of 5, 7 and 9 at 25 °C in a study according to the US EPA 540/9-85-013, Series 161-1 Guideline. The half-lives for photolysis were 33 days at pH 5, 69 days at pH 7 and 77 days at pH 9 in a study carried out according to US EPA 540/9-82-021, Series 161-2 Guideline. In the only ready biodegradation test performed according to OECD TG 301F, glyphosate degraded by < 60% after 28 days. Hence, glyphosate is considered not to be readily biodegradable. In the two inherent degradability tests performed according to OECD TG 302B the substance degraded by 0 % and 2% respectively. Based on the available information on degradation, the DS concluded that glyphosate is not rapidly degradable for classification purposes.

Bioaccumulation

The log Kow for glyphosate acid was < -1.3 in a study according to EEC.A.8 Shake flask method. According to the CLH report, there were no bioaccumulation data available but, as corrected during the PC, one bioconcentration study is presented in the RAR. The BCF (bioconcentration factor) for *Lepomis macrochirus*, in a 56 days flow-through bioconcentration test, was 1.1 ± 0.61. The DS concluded that the potential of glyphosate to bioconcentrate is negligible.

Aquatic toxicity

In the following table, the results of the ecotoxicological tests from acute and chronic studies for three trophic levels are summarised.

Summary of ecotoxicity test results

Test organism / guideline, test method	Short-term result	Long-term result	Reference
Toxicity to fish			
Bluegill Sunfish (<i>Lepomis macrochirus</i>) OECD TG 203/FIFRA 72-1 Static exposure	LC ₅₀ (96h) = 47 mg/L (nom)	-	Kent <i>et al.</i> (1995)
zebra fish larvae (<i>Danio rerio</i>) OECD TG 212 semi-static exposure	-	NOEC (168 h) = 1.0 mg/L (nom) recalculated value key study	Dias Correa Tavares (2000)
Toxicity to aquatic invertebrates			
Acute toxicity to <i>Daphnia magna</i> OECD TG 202 static exposure	LC ₅₀ (48 h) = 84 mg/L (nom) 74 mg/L (meas)		Wüthrich (1990)

Test organism / guideline, test method	Short-term result	Long-term result	Reference
Toxicity to fish			
Acute toxicity to <i>Daphnia magna</i> OECD TG 202 static exposure		NOEC (21 d) = 12.5 mg/L (nom) for reproduction	Magor and Shillabeer (1999)
Toxicity to algae and aquatic plants			
marine alga <i>Skeletonema costatum</i> OECD TG 201 static exposure	ErC ₅₀ (72 h) = 18 mg/L (nom)	NOErC (72 h) = 1.82 mg/L (nom)	Smyth <i>et al.</i> (1996)
blue-green alga <i>Anabaena flos-aquae</i> OECD TG 201 static exposure	ErC ₅₀ (72 h) = 22 mg/L (nom)	NOErC (72 h) = 12 mg/L (nom)	Smyth <i>et al.</i> (1996)
duckweed (<i>Lemna gibba</i>) EPA FIFRA Guidellne 123-2 semi-static exposure	EC ₅₀ (14 d) = 12 mg/L (nom) for inhibition of frond number	NOEC (14 d) = 3 mg/L (nom) for inhibition of frond number	Smyth <i>et al.</i> (1996)

For each test, all the validity criteria according to OECD test guidelines were fulfilled and the studies are considered to be adequate and valid. Where the nominal concentrations are reported, the measured concentrations were between 80 and 120 % of nominal.

The key study for the long-term toxicity classification is based on the OECD TG 212 "Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages". In the test guideline, it is stated that the test should be terminated just before the yolk-sac of any larvae has been completely absorbed. The study was performed for 168 h. RAC highlights that according to the OECD test guideline (annex 3), for *Danio rerio* (zebra fish) the typical duration of the test should be 8-10 days. The DS specifies in the CLH report that in the current test it is not clear if fish in the control treatment are totally free feeding. Despite these deficiencies, the DS considered the study to be valid and acceptable. The NOEC for fish exposed to glyphosate acid was determined by the study author to be 3.2 mg a.s./L based on nominal concentrations. However, at this dose level mortality on larvae of 10 % was observed, clearly following a dose-response relationship. As a consequence the DS concluded that, although not statistically significant, the next lower test concentration should be considered, resulting in a NOEC of 1.0 mg/L.

Comments received during public consultation

Five comments on environmental hazards were received. One MSCA expressed agreement with the proposed classification. One industry organisation, claimed that glyphosate does not meet the classification criteria of a "Long-term (chronic) aquatic hazard because in their opinion the key study used for Long-term (chronic) aquatic toxicity is based on a short-term zebrafish study on sac-fry and fails the validity criteria for a reliable toxicity test for chronic aquatic hazard classification."

According to the DS, the CLH report for glyphosate contains valid and reliable acute and chronic toxicity values from studies for aquatic organisms allowing conclusion on the environmental classification as Aquatic Chronic 2.

One MSCA proposed to take into account several additional studies available on neurotoxicity and genotoxicity in fish for the Chronic classification. Many tests using fish have been conducted in order to investigate the genotoxic and cytotoxic potential of glyphosate towards different aquatic organisms. The DS explained that the cited studies are also reported in the RAR. Referring to biochemical, metabolic and histopathological effects, they were only considered as additional

information, because valid results of aquatic studies with aquatic organisms (including vertebrates) according to standardised test methods (OECD/EU guidelines) or internationally validated and accepted test methods were available. The MSCA also commented on the water/sediment data that was presented in the RAR but excluded from the CLH Report. This data is now added to the RAC opinion under Additional key elements. According to the MSCA, there are different bioaccumulation studies with different aquatic organisms available in the RAR. The BCF values were of max. 10. There is also a literature study available with carp and Tilapia where BCFs ranged from 10 to 65.5.

Assessment and comparison with the classification criteria

A substance is considered to be not rapidly degradable unless at least one of the following is fulfilled:

- a) The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability.
 - Glyphosate degraded < 60% after 28 days in the OECD TG 301 ready biodegradability test thus not reaching the pass level of 60 %.
- b) The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days (corresponding to a degradation of >70 % within 28 days);
 - No study on ultimate degradation in a surface water simulation test is available for glyphosate.
- c) The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydrolysis, in the aquatic environment with a half-life < 16 days (corresponding to a degradation of > 70% within 28 days), and it can be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment.
 - Glyphosate was stable towards hydrolysis. The DT₅₀ values in water/sediment tests were 6.8-21.8 days in the water phase and 13.8-329.9 days in the total system. Adsorption to sediment is a major contributor to the aquatic dissipation of glyphosate. The degradation products AMPA and HMPA do not fulfill the criteria for classification as hazardous to the aquatic environment but degradation half-life < 16 days in the aquatic environment is not demonstrated in these tests.

When evaluating the potential for bioaccumulation experimentally derived BCF values of high quality are ultimately preferred. The BCF for glyphosate in a 56 day flow-through bioconcentration tests with *Lepomis macrochirus* was 1.1 ± 0.61 showing a negligible potential to bioconcentrate. The log Kow for glyphosate acid of < -1.3 also indicates a low potential for bioaccumulation.

Consequently RAC agrees with the DS' conclusion that glyphosate is not rapidly degradable and non-bioaccumulative for the purposes of classification and labelling.

The DS provided short-term and long-term studies for the three trophic levels (fish, invertebrates and algae/aquatic plants). The lowest L(E)C₅₀ obtained for glyphosate is for the aquatic plant *Lemna gibba* (12 mg/L).

According to the criteria of the CLP Regulation, a substance should be classified for aquatic acute toxicity if in an aquatic acute toxicity study, L(E)C₅₀ ≤ 1 mg/L is obtained for any of the three trophic levels fish, invertebrates and algae/aquatic plants. Glyphosate therefore does not fulfil the criteria for classification as Aquatic Acute 1.

Long-term test results for glyphosate are available for three trophic levels (fish, crustacean, algae/aquatic plants). The lowest reliable long-term (chronic) toxicity value is a NOEC = 1 mg/L obtained for fish. Glyphosate is considered not rapidly degradable and therefore fulfils the criteria for classification as Aquatic Chronic 2 (0.1 mg/L < NOEC ≤ 1.0 mg/L).

Based on the additional information on aquatic plant *Myriophyllum aquaticum*, RAC notes that the classification is not necessarily based on an appropriate data set. As a result, the classification might need to be reviewed if further relevant aquatic plant data (e.g. for rooted emergent macrophytes, particularly over long test durations) become available.

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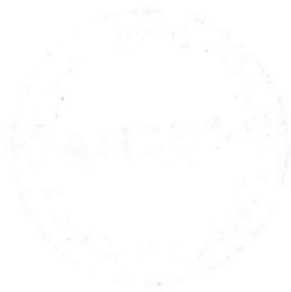
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ANNEXES:

- Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.
- Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).





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Todistetaan Certified	
5. at	Helsingissä Helsinki
6. the	31.5.2018
7. by	Toni Ruotsalainen, Notary Public
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