Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

15 March 2017

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: N-(phosphonomethyl)glycine; Glyphosate (ISO)

EC Number: **213-997-4**

CAS Number: **1071-83-6**

Index Number: **607-315-00-8**

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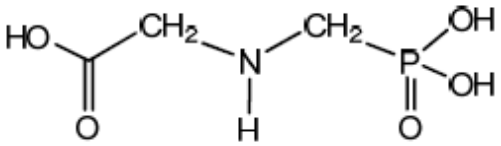
Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*N*-(phosphonomethyl)*glycine*
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	Glyphosate
EC number (if available and appropriate)	213-997-4
EC name (if available and appropriate)	Glyphosate
CAS number (if available)	1071-83-6
Other identity code (if available)	-
Molecular formula	$C_3H_8NO_5P$
Structural formula	
SMILES notation (if available)	C(CN(C[P](O)(O)=O)[H])(O)=O
Molecular weight or molecular weight range	169.1 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 95.0%

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Eye Dam. 1, H318 Aquatic Chronic 2, H411
Current proposal for consideration by RAC	STOT RE 2, H373
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Eye Dam. 1, H318 STOT RE 2, H373 Aquatic Chronic 2, H411

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
2.1.	Explosives				Conclusive but not sufficient for classification
2.2.	Flammable gases				Conclusive but not sufficient for classification
2.3.	Flammable aerosols				Conclusive but not sufficient for classification
2.4.	Oxidising gases				Conclusive but not sufficient for classification
2.5.	Gases under pressure				Conclusive but not sufficient for classification
2.6.	Flammable liquids				Conclusive but not sufficient for classification
2.7.	Flammable solids				Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures				Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids				Conclusive but not sufficient for classification
2.10.	Pyrophoric solids				Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures				Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases				Conclusive but not sufficient for classification
2.13.	Oxidising liquids				Conclusive but not sufficient for classification
2.14.	Oxidising solids				Conclusive but not sufficient for classification
2.15.	Organic peroxides				Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				Conclusive but not sufficient for classification

3.1.	Acute toxicity – oral				Conclusive but not sufficient for classification
	Acute toxicity – dermal				Conclusive but not sufficient for classification
	Acute toxicity – inhalation				Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation				Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Eye Dam. 1, H318		Eye Dam. 1, H318	
3.4.	Respiratory sensitisation				Data lacking
3.4.	Skin sensitization				Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity				Conclusive but not sufficient for classification
3.6.	Carcinogenicity				Conclusive but not sufficient for classification
3.7.	Reproductive toxicity				Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure				Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT RE 2, H373		-	
3.10.	Aspiration hazard				Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Chronic 2, H411		Aquatic Chronic 2, H411	
5.1.	Hazardous to the ozone layer				Data lacking

Labelling: Signal word: Danger
Pictogram: GHS05, GHS08, GHS09
Hazard statements: Causes serious eye damage, May cause damage to organs through prolonged or repeated exposure
Toxic to aquatic life with long lasting effects

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

2.2 Short summary of the scientific justification for the CLH proposal

After evaluation of the available data an additional classification as STOT RE 2 for Glyphosate is proposed based on results obtained in developmental studies in rabbits. Otherwise, the current harmonized classification is confirmed.

2.3 Current harmonised classification and labelling

Eye Dam. 1, H 318;

Aquatic Chronic 2, H 411

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Glyphosate is an active substance in plant protection products. In addition to the existing harmonised classifications for eye irritation and aquatic toxicity, a new classification (STOT RE 2) is proposed.

The re-evaluation of glyphosate as a herbicide by the European Food Safety Authority (EFSA) was required by Commission Regulation (EU) No 1141/2010 as amended by Commission Implementing Regulation (EU) No 380/2013. For this purpose, many new toxicological studies were submitted by the different applicants, especially on eye irritation, genotoxicity, carcinogenicity as well as on reproductive and developmental toxicity of glyphosate. Furthermore, a large number of scientific publications is available and should be considered for the re-evaluation of glyphosate and for the CLH proposal as well. Because of this increase of the toxicological database and also of that one on environmental effects, ECHA and its committee for risk assessment are suggested to address all relevant endpoints.

The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) published in a monograph that glyphosate is “probably carcinogenic to humans (Group 2A)” (IARC, 2015, ASB2015-8421). During the European Food Safety Authority (EFSA) peer-review process for the renewal of approval of the pesticide active substance glyphosate, the IARC evaluation regarding the potential carcinogenicity and genotoxicity of glyphosate or glyphosate -containing plant protection products was taken into consideration but EFSA and EU experts came to a different conclusion (see attached EFSA conclusion, 2015, ASB2015-11412).

The Joint Meeting on Pesticide Residues (JMPR) administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and WHO re-evaluated glyphosate in May 2016 with the following conclusion: “*The Meeting concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures. Several carcinogenicity studies in mice and rats are available. The Meeting concluded that glyphosate is not carcinogenic in rats but could not exclude the possibility that it is carcinogenic in mice at very high doses. In view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet.*” (JMPR, 2016, ASB2016-4292).

Keeping this in mind, the CLH process administered by the European Chemicals Agency (ECHA)

should result in the adoption of a harmonised classification of glyphosate for all health-related but also the environmental endpoints.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

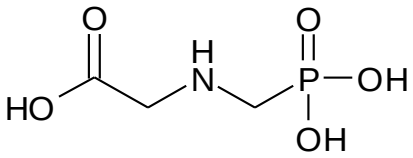
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	213-997-4
EC name:	Glyphosate
CAS number (EC inventory):	1071-83-6
CAS number:	1071-83-6
CAS name:	N-(phosphonomethyl)-glycine
IUPAC name:	N-(phosphonomethyl)-glycine
CLP Annex VI Index number:	607-315-00-8
Molecular formula:	C3H8NO5P
Molecular weight range:	169.1 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
N-(phosphonomethyl) glycine	≥ 95.0%	≥ 95.0%	

Table 6: Impurities (non-confidential information)

Impurity	**Typical concentration**	**Concentration range**	**Remarks**
N-Nitroso-glyphosate	< 1 ppm	< 1 ppm	This value was decreased by the RMS based on the toxicological evaluation
Formaldehyde	< 1 g/kg	< 1 g/kg	This value was decreased by the RMS based on the toxicological evaluation

Table 7: Additives (non-confidential information)

Additive	**Function**	**Typical concentration**	**Concentration range**	**Remarks**
-				

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid, crystalline powder	Hammond and Pulwer, 1986	Measured
Melting/freezing point	> 200 °C (decomposition)	Wollerton and Husband, 1997	Measured
Boiling point	> 200 °C (decomposition)	Wollerton and Husband, 1997	Measured
Relative density	d_4^{20} = 1.7018	Wollerton and Husband, 1997	Measured
Vapour pressure	< 10^{-5} Pa (20 °C)	Wollerton and Husband, 1997	Measured
Surface tension	72.7 mN/m (1 g/L in dist. H_2O, 20 °C)	Wollerton and Husband, 1997	Measured
Water solubility	10 g/L, EEC A 6 flask method	Wollerton and Husband, 1997	Measured
Partition coefficient n-octanol/water	log $P_{o/w}$ < - 1.3 EEC A 8 shake flask	Wollerton and Husband, 1997	Measured
Flash point	not required		
Flammability	not highly flammable under the conditions of the test (EEC A 10)	Wollerton and Husband, 1997	Measured
Explosive properties	not explosive	Wollerton and Husband, 1997	theoretical assessment
Self-ignition temperature	not auto-flammable (EEC A 15)	Wollerton and Husband, 1997	Measured
Oxidising properties	non-oxidising	Wollerton and Husband, 1997	Measured
Granulometry	No data	-	-
Stability in organic solvents and identity of relevant degradation products	No data	-	-
Dissociation constant	pK_{a1} = 2.25 (20 °C) pK_{a2} = 5.50 pK_{a3} = 10.34 OECD 112 titration	Wollerton and Husband, 1997	Measured
Viscosity	No data	-	-

2 MANUFACTURE AND USES

Glyphosate is a non-selective post-emergence, mono- and dicotyledonous herbicidal active substance.

3 SUBSTANCECLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not addressed in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

The main data source for the evaluation of the toxicological properties of glyphosate with regard to classification and labelling was the revised Renewal Assessment Report (RAR) dated 31 March 2015, which was written for the EU pesticides procedure. Volumes 1 and 3 are attached to the CLH dossier as background documents. This version was produced after discussion of the draft RAR of the Rapporteur Member State (RMS) Germany on an expert meeting (PRAS) hold by EFSA in February, 2015, and reflects the conclusions drawn there. The only classification that was agreed at that time was for eye irritation. Thus, it should be acknowledged that the additional German proposal for classification (STOT RE 2) has been made after that meeting and, thus, was not subject to commenting by Member States or expert meeting discussion so far. Going beyond the RAR, a number of additional long-term, reproduction and developmental studies are addressed in this CLH dossier that were found unsuitable for risk assessment purposes and, therefore, have been rejected during the EU re-evaluation process although some of them may have been used for a previous one. Even if the deficiencies in these studies do not have an impact on classification and labelling, they are at least briefly mentioned to ensure that a comprehensive picture for these endpoints is provided. With regard to genotoxicity/mutagenicity, we have included studies that do not comply with current standards only if they revealed a positive result which needed to be addressed.

Another important basis for the current evaluation is a new assessment of the International Agency for Research on Cancer (IARC) to assign glyphosate to category 2A for carcinogenicity. IARC's decision was published in July, 2015, when the IARC Monograph 112 was released. The assessment of this monograph in an addendum to the RAR by the German Federal Institute for Risk Assessment (BfR) has been completed on 31 August 2015 and was submitted in September, 2015, to EFSA as an addendum to the RAR. This addendum has been subject to thorough peer review by the competent authorities of the EU Member States. During this review process, including an expert discussion held by EFSA on 29 September 2015, all the Member States experts but one agreed that the active substance is unlikely to be genotoxic or to pose a carcinogenic threat to humans and is not proposed to be classified as such under EU regulations. The addendum and the EFSA documentation are also attached to this CLH dossier to provide background information.

All toxicological studies included in this CLH dossier were evaluated and assessed by in-house staff toxicologists of the BfR. It is emphasised that the toxicological database for glyphosate is extremely large and that the studies have come from a great number of sources. Thus, completeness of the database and identification and compilation of relevant and reliable data are crucial. In the following, the approach taken by the dossier submitter (DS) is described with particular regard to the studies and publications that are referred to in this CLH dossier.

The information that is relevant for classification and labelling of glyphosate is based on original studies of the manufacturers that were performed on a routine basis under GLP conditions and in compliance with OECD Test Guidelines for the individual toxicological endpoints. Such studies are usually confidential and are submitted to national authorities or supranational bodies to support authorisation or registration of plant protection products containing the respective active ingredient.

In case of glyphosate, these studies have been reported in detail in the RAR. Nonetheless, most of them have not been made publically available in full and they would not been found in a systematic literature review since they are proprietary to their owners.

A further source of information is published literature. For classification and labelling purposes, mainly epidemiological studies have been taken into consideration whereas there were only few published *in vivo* or *in vitro* studies with the active substance glyphosate. It must be emphasised that in most of these studies formulations of glyphosate instead of the active substance have been tested.

(1) The search for published studies was based on: The scientific literature concerning glyphosate, its salts, AMPA and also glyphosate formulations with regard to side effects on health, the environment, and non-target species as provided by the "Glyphosate Task Force" (GTF) (Carr and Bleeke, 2012, ASB2012-11583). The period from 2001 to 2011 was covered. The search was performed in five databases: Web of Science, BIOSIS Previews, CAB Abstracts, CA Plus (Chemical Abstracts Plus), and Medline.

(2) A dossier on glyphosate submitted by various non-governmental organizations (NGOs) containing further references even though a part was overlapping with the manufacturer's search.

(3) Several new publications that became available before, during and after the commenting phase of the RAR (including the "public consultation").

(4) A check of the reference lists of the submitted articles by the DS for so far unknown references.

This section contains short summaries and purpose-adapted tables frequently adopted and taken from the RAR as well as from the addendum. In case more in-depth information on the studies and effects is needed, the reader is referred to Vol. 3, chapter B.6 of the RAR where all the studies are reported in detail. Most toxicological studies were performed on behalf of various manufacturers with technical specifications from many sources. Accordingly, the purity and impurity profile were different. Impurities may have contributed to the toxic effects but there is no data to determine the extent of this contribution. In the European context this has led to the situation that a number of specifications from different applicants were not supported by the toxicological assessment (see attached EFSA conclusion, 2015, ASB2015-11412).

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human data

Experimental studies in laboratory animals (mainly rats) are available in which toxicokinetics and metabolism (ADME) of glyphosate have been investigated. The understanding of toxicokinetics and metabolism of a chemical is considered as crucial for its toxicological evaluation.

Glyphosate is rapidly absorbed from the gastro-intestinal tract (GIT) following oral intake but only to a limited extent of about 20%. It is widely distributed to the various compartments, organs and tissues. Elimination is fast and virtually complete within 72-168 hours with the major part being excreted already during the first 48 hours. The absorbed part is excreted in the urine whereas the (greater) unabsorbed portion is eliminated via the faeces. Enterohepatic circulation and biliary excretion are negligible, and so is exhalation. After a period of 3 to 7 days following oral administration, total body burden accounted for ≤1% of the applied radioactivity with generally low tissue residues at study termination (Ridley and Mirly, 1988, TOX9552356; Powles & Hopkins, 1992, TOX9300343; Davies, 1996, TOX2000-1977, TOX2000-1978, TOX2000-1979; McEwen, 1995, ASB2012-11379; Knowles and Mookherjee, 1996, ASB2012-11380). Highest residues were detected

in bone, followed by kidney and liver. Due to poor oral absorption, high amounts were also found in the GIT. This pattern of distribution was confirmed by whole-body autoradiograms that showed the greatest intensity of radioactivity to be present in bone and the gastrointestinal tract not later than 24 hours after dosing. These amounts were reduced to negligible amounts within 48 hours (Powles and Hopkins, 1992, TOX9552358; Davies, 1996, TOX2000-1980). Although elimination from bone seems slower than from other tissues, the amount of radiolabel in bone tissue at 168 h after a single oral dose was relatively low accounting for not more than 0.02-0.03% of the applied dose (McEwen, 1995, ASB2012-11379).

There was no evidence of accumulation in animals based on residue analysis in organs and tissues at 72-168 h after single or repeated doses.

This pattern of absorption, distribution and elimination was not significantly changed by dose levels or by repeated administration of low doses and was independent of the sex of the test animals.

Most of the parent substance glyphosate was eliminated unchanged and only a small amount (in most studies less than 1% of the applied dose and sometimes none) was transformed to aminomethylphosphonic acid (AMPA). There is only one publication by Anadon et al. (2009, ASB2012-11542) that suggests a higher metabolism rate of up to 6.5% of the dose following oral administration of 400 mg/kg bw to rats. Formation of AMPA is assumed to be due to gastrointestinal microflora activity rather than mammalian metabolic pathways (Brewster et al., 1991, TOX9551791). AMPA was broadly investigated for many toxicological endpoints and exhibited similar or lower toxicity than glyphosate and was found to be devoid of genotoxic potential (see RAR). The same reference doses as for glyphosate are applicable.

In Table 9 the acceptable ADME studies with glyphosate and their results are compiled.

Table 9: Comparison of the distribution of radiolabelled glyphosate acid in excreta and tissues and its metabolism in valid ADME studies in the rat

Reference, Study identification, Owner	**Dosing regime and dose levels, Duration of post-observation period**	**Excretion / Distribution (mean % of applied dose)**								**Metabolism**
		Urine		**Faeces**		**Total organ / tissue / carcass residues**		**Bile**		
		♂	♀	♂	♀	♂	♀	♂	♀	
Leuschner (1995)#, TOX9650071 / Blech & Stratmann (1995) #, TOX9552251; ADAMA	0.2-0.3 mg/kg bw, single oral dose, 168 h	12.3	9.6	82.9	83.3	--	--	--	--	No metabolites found in urine following oral high dose application
	200 mg/kg bw, single oral dose, 168 h	17.1	13.2	81.8	84.4	--	--	--	--	
	0.2 mg/kg bw, single i.v. dose, 168 h	90	88.6	5.6	7.2	< 0.1*	< 0.1*	--	--	
Powles & Hopkins (1992), TOX9300343;	30 mg/kg bw, single oral dose, 168 h	29.0	30.7	58.8	56.5	0.62	0.64	--	--	No metabolites found in urine or faeces
	1000 mg/kg bw, single	30.6	22.4	53.3	60.4	0.47	0.40	--	--	

Reference, Study identification, Owner	Dosing regime and dose levels, Duration of post-observation period	Excretion / Distribution (mean % of applied dose)								Metabolism
		Urine		Faeces		Total organ / tissue / carcass residues		Bile		
		♂	♀	♂	♀	♂	♀	♂	♀	
Cheminova	oral dose, 168 h									
	30 mg/kg bw, repeated (14x) oral application followed by a single radiolabelled dose, 72 h	34.3	34.6	49.6	46.7	0.96	0.83	--	--	
	30 mg/kg bw, single i.v. dose, 168 h	86.0	84.2	3.4	1.5	1.4	1.1	--	--	
Ridley & Mirly (1988), TOX9552356 / Howe et al. (1988), TOX9552357; Monsanto	10 mg/kg bw, single oral dose, 168 h	28.6	22.5	62.4	69.4	0.48	0.36	--	--	Very limited, AMPA accounting for 0.2-0.4%
	1000 mg/kg bw, single oral dose, 168 h	17.8	14.3	68.9	69.4	<0.4	<0.4	--	--	
	10 mg/kg bw, repeated (14x) oral application followed by a single radiolabelled dose, 168 h	30.9	23.1	61.0	70.9	<0.7	<0.7	--	--	
	10 mg/kg bw, single i.v. dose, 168 h[$]	79.0	74.5	4.7	8.3	≈ 1.0	≈ 1.0	--	--	
McEwen (1995), ASB2012-11379; Arysta	Single oral gavage, 168 h; satellite groups for plasma kinetics									Very limited, traces of AMPA in urine (<0.3%) and of AMPA and another compound in faeces (<2%)
	10 mg/kg bw	22.5	19.4	74.6	84.3	0.33	0.27	--	--	
	600 mg/kg bw	30.3	29.5	74.7	74.2	0.31	0.39	--	--	
Knowles & Mookherjee (1996), ASB2012-11380;	Single oral gavage, 168 h; satellite groups for plasma kinetics and									Very limited with <1% transformed to a compound presumed as AMPA

Reference, Study identification, Owner	Dosing regime and dose levels, Duration of post-observation period	Excretion / Distribution (mean % of applied dose)								Metabolism
		Urine		Faeces		Total organ / tissue / carcass residues		Bile		
		♂	♀	♂	♀	♂	♀	♂	♀	
Nufarm	tissue residues (up to 72 h) and 48-h biliary excretion									
	1 mg/kg bw	24.9	34.9	72.6	62.4	0.75	0.98	--	--	
	100 mg/kg bw	55.3	55.0	41.2	42.4	0.84	0.98	--	--	
	1 mg/kg bw	27.5	24.2	55.3	61.0	4.99	3.82	0.03	0.08	
Macpherson (1996), TOX2000-1981; Syngenta	Single oral gavage, 1000 mg/kg bw, 48 h	20.8	16.3	39.1	30.5	--	--	0.06	0.06	Very limited, <0.7% AMPA was found (based on examination of urinary and faecal samples obtained over 72 hours in other experiments from the same lab, i.e., Davies, 1996a-c)
Davies (1996a), TOX2000-1977; Syngenta	Single oral gavage, 10 mg/kg bw, 72 h	13.3	11.1	88.5	88.7	0.54	0.46	--	--	Not investigated
Davies (1996b), TOX2000-1978; Syngenta	Single oral gavage, 1000 mg/kg bw, 72 h	16.9	17.8	89.5	84.6	0.47	0.54	--	--	Not investigated
Davies (1996c), TOX2000-1979; Syngenta	Single oral dose (gavage) after repeated (14x) dosing, 10 mg/kg bw, 72 h (after final dose)	10.6	10.7	86.8	90.7	0.47	0.41	--	--	Not investigated

[#] Supplementary study. * Bone tissue not investigated. [$] Total recovery was rather poor.

In addition, there is a rather old (supplementary) study with dietary administration of glyphosate over 14 days to rats (Colvin and Miller, 1973, TOX9552355) where evidence of even a lower oral absorption than after gavage application was obtained. Total excretion was found to equal total intake. A supplementary study in male rabbits (Colvin and Miller, 1973, TOX9552353) demonstrated a similar pattern of toxicokinetics and metabolism as in the rat.

Following dermal exposure to rabbits, glyphosate was poorly (< 3%) absorbed (Hadfield, 2012,

ASB2012-11459) but the actual extent of dermal absorption depends very much on the product in which the active ingredient is formulated.

4.1.2 Human data

Reliable kinetic data obtained in humans are not available for glyphosate. However, based on an analysis of a total of 13 poisoning incidents with glyphosate-based herbicides in France (Zouaoui et al., 2013, ASB2014-9734), there is at least strong evidence that biotransformation of ingested glyphosate to AMPA is very limited also in man. The glyphosate:AMPA ratio in blood analyses varied between 12:1 and 6933:1 with a median value of 235:1. In urine, with data from 7 cases available, the individual ratios ranged from 243:1 to 7863:1 with a median of 422:1. These ratios were independent from the severity of symptoms or a fatal outcome.

4.2 Acute toxicity

4.2.1 Non-human information

A huge number of acute oral, dermal and inhalation studies with glyphosate is available. In the majority of experiments, the test species was the rat. A few studies have been conducted in other animal species such as the mouse suggesting that they were not more vulnerable than the rat after oral administration. The available data is compiled in Table 10, Table 11, and Table 12 and briefly summarised below for each route.

Acute oral toxicity

Table 10: Summary of acute oral toxicity studies with glyphosate acid in rats and mice

Reference, (Owner), Study identification	Species, Strain	Number of animals / dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Sharp, 1995 (Sanachem) TOX9650909	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	>2000 (limit test)	Slightly congested lungs, splenomegaly, Liver: centri-lobular congestion
Snell, 1994 (Herbex) TOX9500245	Rat, Sprague Dawley	1/sex/2000 5/sex/2000	95	Arachis oil	>2000 (limit test)	No findings
Tornai et al., 1994 (Alkaloida) TOX9650142	Rat, Wistar	5/sex/0 5/sex/5000	97.2	Water	>5000 (limit test)	♂: heart weights↓
Brown and Ogilvie, 1995 (Sinon) TOX9500377	Rat, Sprague Dawley	2/sex/250 2/sex/500 2/sex/1000 2/sex/3000 2/sex/5000 5/sex/5000	95	CMC	>5000 (limit test)	Piloerection, subdued behaviour, hunched appearance
Walker and Jones, 1992 (Barclay)	Rat, Sprague Dawley	1/sex/2000 5/sex/2000	>97	Water	>2000 (limit test)	No findings

Reference, (Owner), Study identification	Species, Strain	Number of animals / dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
TOX9551810						
Suresh, 1991 (Feinchemie, now ADAMA) TOX9551088	Rat, Wistar	5/sex/2500 5/sex/5000 5/sex/7500	96.8	Peanut oil	>7500 (estimated)	7500 mg/kg bw: mortality (2/5 ♂, 2/5 ♀); lethargy, ataxia, dyspnoea, weight loss
Brett, 1990 (Agrichem) TOX9500261	Rat, CD	5/sex/0 5/sex/3000 5/sex/5000 5/sex/8000	98.1	1% CMC	>8000	≥5000 mg/kg bw: decreased activity, abnormal gait and/or limb position
Cuthbert & Jackson, 1989 (Cheminova) TOX9552319	Rat, Sprague Dawley	5/sex/5000	98.6	0.5% CMC	>5000 (limit test)	Piloerection, reduced activity, ataxia (♂ only)
You, 2009 (Helm) ASB2012-11381	Rat, Sprague Dawley	5/females/5000	96.4	Water	>5000 (limit test)	Decreased activity, diarrhoea, piloerection, polyuria, salivation
Komura, Hitoshi, 1995 (Arysta) ASB2012-11382	Rat, Sprague Dawley	5/sex/5000	95.68	0.5% CMC	>5000 (limit test)	Decreased spontaneous motor activity and salivation
Simon, 2009 (Exxel) ASB2012-11384	Rat, Wistar	3 females/2000 (step 1) 3 females/2000 (step 2)	96.66	Water	>2000	No findings
Haferkorn, 2009 (Helm) ASB2012-11385	Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	98.8	0.8% hydro-xypro-pylme-thylcel-lulose	>2000 (limit test)	No findings
Haferkorn, 2010 (Helm) ASB2012-11386	Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	96.4	0.8% hydro-xypro-pylme-thylcel-lulose	>2000 (limit test)	No findings
Haferkorn, 2010 (Helm) ASB2012-11387	Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	97.3	0.8% hydro-xypro-pylme-thylcel-lulose	>2000 (limit test)	No findings
Merkel, 2005a (Helm) ASB2012-11388	Rat, Sprague-Dawley	3 females/5000	97.23	Water	>5000 (limit test)	Diarrhea, ano-genital & facial staining, reduced faecal volume
Do Amaral	Rat, Wistar	3 females/2000	98.05	Water	>2000	No findings

Reference, (Owner), Study identification	Species, Strain	Number of animals / dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Guimaraes 2008 (Helm) ASB2012-11389		(step 1) 3 females/2000 (step 2)			(limit test)	
Taivioja, 2007 (Nufarm) ASB2012-11390	Rat, HanRcc:WIST	2 x 3 ♀/2000	95.1	PEG 300	>2000 (limit test)	Slightly ruffled fur
Reagan and Laveglia, 1988 (Monsanto) Z35389	Rat, Sprague Dawley	5/sex/5000	97.76	Water	>5000	Diarrhea, apparent urinary incontinence and hair loss on the abdomen
Heenehan et al., 1979 (Monsanto) Z35541	Rat, Wistar	5/sex/2500 5/sex/3500 5/sex/5000 5/sex/7000 5/sex/9900	99	Water	>5000	Mortalities: 1/10 1/10, 3/10,7/10, 10/10 at 2500, 3500, 5000, 7000 and 9900 mg/kg bw; clinical signs: ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and fecal staining of the abdomen
Doyle, 1996 (Syngenta) TOX2000-1982	Rat	5/sex/5000	95.6	Water	>5000	No findings
Arcelin, 2007 (Syngenta) ASB2012-11391	Rat	3 ♀/5000	96.1	Water	>5000	Ruffled fur, hunched posture
Tavaszi, 2011 (Syngenta) ASB2012-11392	Rat	3 ♀/5000	96.3	0.5% CMC	>5000	No findings
Pooles, 2014 (Albaugh Europe Sàrl) ASB2014-9147	Rat	5 ♀/2000	85.8	DMS	>2000 (fixed dose method)	Hunched posture
Komura, Hitoshi, 1995 (Arysta) ASB2012-11383	Mouse, ICR	5/sex/5000	95.68	0.5% CMC	>5000 (limit test)	Decreased spontaneous motor activity, sedation and crouching position
Suresh, 1991 (FSG, now ADAMA) TOX9551089	Mouse, Swiss albino	5/sex/2500 5/sex/5000 5/sex/7500	96.8	Peanut oil	>7500	≥2500 mg/kg bw: mortality, lethargy, ataxia, dyspnoe, weight loss

Reference, (Owner), Study identification	Species, Strain	Number of animals / dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Tos et al., 1994 (Industria Prodotti Chimici) TOX9551624	Mouse, Charles River	5/sex/2000	technical	0.5% CMC	>2000 (limit test)	Piloerection, hunched posture, hypoactivity
Dideriksen & Skydsgaard 1991 (Cheminova) TOX9552320	Mouse, Bom:NMRI	5/sex/2000	98.6	Water	>2000 (limit test)	Piloerection, sedation

CMC = carboxymethylcellulose

Frequently occurring signs of oral intoxication were breathing difficulties, diarrhea, reduced activity, ataxia, piloerection, convulsions and hunched posture. Mortality was seen in few studies only and was confined to very high dose levels. The lowest dose causing mortality was 2500 mg/kg bw as reported by Suresh (1991, TOX9551089) for the mouse and by Heenehan et al. (1979, Z35541) for the rat. The number of dead animals at this dose was low and many studies have demonstrated that most animals tolerated the same or much higher doses of 5000 mg/kg bw or even above. Since the oral studies in rats and mice consistently revealed LD_{50} values >2000 mg/kg bw, classification for acute oral toxicity according to CLP regulation is not required.

Acute dermal toxicity

Table 11: Summary of acute dermal toxicity studies with glyphosate acid on rats and rabbits

Reference, (Owner,) Study identification	Species Strain	Number of animals/ Dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Sharp, 1995 (Sanachem) TOX9650910	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	>2000 (limit test)	Splenomegaly, Liver: centri-lobular congestion
Meyer-Carrive, 1994 (Sinon) TOX9500378	Rat, Sprague Dawley	5/sex/2000	95	Suspen-ded (50% w/w) in natrosol (1% w/w in water)	>2000 (limit test)	No findings
Snell, 1994 (Herbex) TOX9500246	Rat, Sprague Dawley	5/sex/2000	95	None	>2000 (limit test)	No findings
Tornai et al, 1994 (ALkaloida) TOX9650143	Rat, Wistar	2/sex/0 5/sex/2000	97.2	Water	>2000 (limit test)	No findings
Walker, 1992 (Barclay) TOX9551813	Rat, Sprague-Dawley	5/sex/2000	> 97	None	>2000 (limit test)	No findings
Suresh, 1991 (FSG, now ADAMA) TOX9551090	Rat, Wistar	5/sex/2500 5/sex/5000	96.8	Water (slurry)	>5000	body weight loss
Brett, 1990 (Agrichem) TOX9551793	Rat, CD	5/sex/0 5/sex/3000 5/sex/5000 5/sex/8000	98.1	0.9% saline	>8000	No findings
Cuthbert & Jackson, 1989 (Cheminova) TOX9300328	Rat, Sprague Dawley	5/sex/2000	98.6	Water for moiste-ning	>2000 (limit test)	No mortalities, body weight loss in one female, scab formation at application site; 0.5 h-1d after dosing reduced activity and piloerection
You, 2009 (Helm) ASB2012-11395	Rat, Sprague Dawley	5/sex/5050	96.4	Water	>5050	body weight loss in 1 male and 1 female
Komura, Hitoshi, 1995 (Arysta) ASB2012-11396	Rat, SD	5/sex/2000	95.68	Water	>2000 (limit test)	No findings
Simon, 2009 (Exxel) ASB 2012-11397	Rat, HanRcc:WIST	5/sex/2000	96.66	Water	>2000	No mortalities, no signs of systemic toxicity; in 4 females slight local signs (erythema, scaling and scabs) at the application sites

Reference, (Owner,) Study identification	Species Strain	Number of animals/ Dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Haferkorn, 2009 (Helm) ASB2012-11398	Rat, CD	5/sex/2000	98.8	Water	>2000	No findings
Haferkorn, 2010 (Helm) ASB2012-11399	Rat, CD	5/sex/2000	96.4	Water	>2000	No findings
Haferkorn, 2010 (Helm) ASB2012-11400	Rat, CD	5/sex/2000	97.3	Water	>2000	No findings
Merkel, 2005 (Helm) ASB2012-11401	Rat, Sprague Dawley	5/sex/5000	97.23	Water	>5000	No findings
Do Amaral Guimaraes 2008 (Helm) ASB2012-11402	Rat, Wistar Hannover	5/sex/2000	98.05	Water (for moisto-ning)	>2000	No findings
Taivioja, 2007 (Nufarm) ASB2012-11403	Rat, HanRcc:WIST	5/sex/2000	95.1	PEG 300	>2000 (limit test)	No findings
Doyle, 1996 (Syngenta) TOX2000-1983	Rat	5/sex/2000	95.6	Moiste-ned with deionised water	>2000	Slight erythema in 1♂, small scabs in 1 ♀
Arcelin, 2007 (Syngenta) ASB2012-11404	Rat	5/sex/5000	96.1	Moiste-ned with purified water	>5000	No findings
Zelenak, 2011 (Syngenta) ASB2012-11405	Rat	5/sex/5000	96.3	Moiste-ned with purified water	>5000	No findings
Reagan and Lavveglia, 1988 (Monsanto) TOX9552325	Rabbit, NZW	5/sex/5000	97.8	Moiste-ned with saline	>5000	Mortality (1 ♀); anorexia, diarrhea, soft stool

Apart from one female rabbit receiving 5000 mg/kg bw (Reagan and Lavveglia, 1988, TOX9552325), there were no deaths. Isolated signs of toxicity comprised body weight loss, diarrhea and slight local effects. Overall, the dermal studies with glyphosate acid in rats and rabbits revealed LD_{50} values of >2000 mg/kg bw or even of >5000 mg/kg bw. Therefore, classification for acute dermal toxicity according to CLP regulation is not required.

Acute inhalation toxicity

Table 12: Summary of acute inhalation toxicity studies with glyphosate acid

Reference, (Owner,) Study identification	Species Strain	Number of animals / Concentrations (mg/L air)	Purity (%)	Exposure conditions; Particle size if given	LC_{50} (mg/L air)	Main effects
Blagden, 1995 (Herbex) TOX9500247	Rat, Sprague Dawley	5/sex/5.35	95	Compressed air; 4 h nose-only	>5.35	Wet fur, hunched posture, piloerection, incidents of decreased respiratory rate, ptosis, brown stained fur (head)
Tornai, 1994 (Alkaloida) TOX9650144	Rat, Wistar	5/sex/0 5/sex/1.138 5/sex/2.876	97.2	Watery aerosol; 4 h exposure, route not stated	>2.876	Trachea: lymphoid cell infiltration, mucous lung: congestion, haemorrhages, oedema liver: mononuclear cell infiltrations, congestion kidney: congestion, nephrocalcinosis
McDonald & Anderson, 1989 (Cheminova) TOX9552329	Rat, Sprague Dawley	5/sex/4.98	98.6	Dust aerosol; 4 h snout only	>4.98	No adverse findings
Haferkorn, 2010 (Helm) ASB2012-11406	Rat, CD	5/sex/5.18	97.3	4 h nose only (MMAD: 4.63 µm)	>5.18 (limit test)	Slight tremor, slight dyspnoea
Koichi, 1995 (Arysta) ASB2012-11407	Rat, Fischer F344	5/sex/5.48	97.56	Dust,4 h whole body (MMAD: 4.8 µm)	>5.48	Wet and soiled fur (periocular and nasorostral)
Griffith, 2009 (Exxel) ASB2012-11408	Rat	5/sex/5.04	96.66	Dust, 4 h, nose-only, (MMAD 5.25 µm)	>5.04	Increased respiratory rate, hunched posture, pilo-erection, wet fur
Haferkorn, 2009 (Helm) ASB2012-11409	Rat, CD	5/sex/5.12 (dust)	98.8	4h (MMAD: 6.62 µm)	>5.12 (limit test)	Slight dyspnoea and ataxia during exposure
Haferkorn, 2010 (Helm) ASB2012-11410	Rat, CD	5/sex/5.02	96.4	4h (MMAD: 4.2 µm)	>5.02	Slight dyspnoea, slight ataxia and slight tremor during exposure until 3 h after exposure
Carter, 2009 (Helm) ASB2012-11411	Rat, Sprague-Dawley	5/sex/2.24	96.4	4 h (MMAD: 2.6 µm)	>2.24 (limit test)	No findings
Merkel, 2005 (Helm) ASB2012-11412	Rat, Sprague-Dawley	5/sex/2.04	97.23	4 h (MMAD: 2.5 µm)	>2.04 (limit test)	No findings
Decker, 2007 (Nufarm) ASB2012-11414	Rat, albino	5/sex/3.252	95.1	4 h (MMAD: 2.95-3.05 µm)	> 3.252	Salivation in males, breathing effects in both sexes, body

Reference, (Owner,) Study identification	Species Strain	Number of animals / Concentrations (mg/L air)	Purity (%)	Exposure conditions; Particle size if given	LC_{50} (mg/L air)	Main effects
						weight loss
Rattray, 1996 (Syngenta) TOX2000-1984	Rat	5/sex/4.43 5/sex/2.47	95.6	4 h, nose-only, (MMAD: 2.91 and 3.41 µm)	>4.43	Mortality: 2♂ & 2♀ at 4.43 mg/L. Irregular breathing, splayed gait, shaking & reduced righting reflex
Nagy, 2011 (Syngenta) ASB2012-11415	Rat	5/sex/5.04	96.9	4 h nose-only (MMAD: 3.65 µm)	>5.04	Mortality: 1♂ on day 4. Laboured and noisy respiration, respiratory rate increase, gasping respiration, sneezing, decreased activity and thin body appearance observed until day 3.

Inhalation toxicity of glyphosate was tested in rats and consistently found to be low. In many studies, a concentration ≥5 mg/L was tested. Thus, information on effects of inhaled glyphosate at high concentrations is sufficient even though this limit concentration was not attained in all experiments. Various clinical signs such as 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 were observed but were not consistent among the studies. Mortality was confined to the experiments of Rattray (1996, TOX2000-1984) and Nagy (2011, ASB2012-11415) using both test material of the same manufacturer but did not result in an LC50 value below 5 mg/L. Both studies are reported in detail in Volume 3 of the RAR in sub-section B.6.2.3. Since classification for inhalation toxicity is usually based on the LC_{50}, there is no need to classify glyphosate for this endpoint according to the CLP regulation since 5 mg/L air is the trigger concentration for dusts and mists.

4.2.2 Human data

No studies or case reports are available in which humans would have been exposed to the active ingredient itself. However, over the course of time, a number of poisoning incidents have been reported that were due to accidental or intentional (mostly oral, in very few cases inhalative) intake of glyphosate-based herbicides. For summary, see Vol.1, Section 2.6.11, and Vol. 3, B.6.9.4, of the attached RAR. In most cases, actual exposure remained unknown. Furthermore, it is not possible to clearly distinguish between effects due to glyphosate and those caused by co-formulants.

A calculation of ingested doses in a few cases of severe intoxications, including fatalities, suggests that a potentially lethal dose of glyphosate contained in plant protection products to humans will be above 2000 mg/kg bw. According to Lee et al. (2000, ASB2012-11512), Beswick and Millo (2011, ASB2014-9283), Sribanditmongkol et al. (2012, ASB2014-9731) or Zouaoui et al. (2013, ASB2014-9734), ingestion of 300 mL or more of products such as Roundup® containing 36 to 41% glyphosate may result in a fatal outcome, even though most patients survived. A dose of 300 mL of such a formulation would contain up to 123 g glyphosate resulting in a dose of ca 2050 mg/kg bw in a man weighing 60 kg. There is strong evidence that certain co-formulants, e.g., some polyoxethylated alkylamines (POEA, used as surfactants), may either enhance the toxicity of glyphosate or exhibit independent toxic properties resulting in a higher toxicity of many formulations as compared to the

active ingredient (see Vol. 3, B.6.13.3). As far as is known, such surfactants were part of the plant protection products that were ingested in the described clinical cases.

On balance, a higher acute toxicity of glyphosate to humans than to rats is not likely.

Accordingly, poisoning incidents in humans do not support classification and labelling of glyphosate for acute toxicity and are not appropriate for this purpose.

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 $\geq$ 5000 mg/kg bw. Since the LD_{50} 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 $\geq$ 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 LC_{50} value in these studies was $\geq$ 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 LD_{50} values > 2000 mg/kg bw and 10 with an LD_{50} value of > 5000 mg/kg bw. In the remainder of the acute toxicity tests the LD_{50} values ranged from >5000 to > 8000 mg/kg bw. Three acute oral toxicity studies were performed in mice as limit tests with LD_{50} values > 2000 mg/kg bw. In the fourth acute toxicity test in mice an LD_{50} 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, LD_{50} 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 LC_{50} 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 LC_{50} values > 2.0 mg/L and one study with an LC_{50} value of > 3.25 mg/L. Two studies had two concentrations of glyphosate with LC_{50} 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 LC_{50} values below 5.0 mg/L. Both studies used glyphosate from the same source.

RAC concludes that following inhalation exposure to glyphosate no LC_{50} 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.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Non-human information

Based on the multitude of acute toxicity studies in rats and mice (see Table 9, Table 10, and Table 11), classification of STOT SE (categories 1 or 2) is not appropriate because non-lethal effects were confined to very high doses and were rather unspecific. This assessment is further supported by the acute neurotoxicity study in rats (Horner, 1996, ASB2012-11500, see Vol. 3, B.6.7) in which no evidence of neurotoxicity was observed at dose levels of 500, 1000, and 2000 mg/kg bw even though unspecific clinical signs occurred and one single female animal was found dead at the top dose level. No clinical evidence of single (i.e., first) dose effects was obtained from the many toxicological studies with repeated administration in which lower doses were applied. Suitable haematological and clinical chemistry data is not available since sampling was not performed during the first days of treatment but, taking into account the toxicological profile of glyphosate, alterations in these parameters are not expected.

With regard to category 3, no evidence of narcotic effects was obtained in any toxicological study. For considerations of respiratory tract irritation, the reader is referred to 4.4.3.

In summary, there is no need to classify glyphosate for STOT SE.

4.3.2 Human data

No appropriate data is available for the active substance. No evidence of organ-specific non-lethal effects (except eye irritation) can be derived from poisoning incidents with formulations.

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 (POEA) 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.

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4.4 Irritation

4.4.1 Skin irritation

In older studies (see Vol. 3, B.6.2.4), either no or only slight/very slight irritation was found. A number of more recent, guideline-compliant studies in rabbits have been submitted for the new EU evaluation and are summarised in Table 13.

Table 13: Summary of most recent skin irritation studies with glyphosate acid

Study (Owner)	Species Strain	Number and sex of animals	Purity [%]	Amount applied / Exposure conditions	Result
Talvioja, 2007 (Nufarm) ASB2012-11418	Rabbit NZW	1 ♂, 2 ♀	95.1	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
Hideo, 1995	Rabbit	6 ♀	97.56	0.5 g moistened with	Non irritant

Study (Owner)	Species Strain	Number and sex of animals	Purity [%]	Amount applied / Exposure conditions	Result
(Arysta) ASB2012-11420	NZW			0.5 mL water; intact skin	
Leuschner, 2009a (Helm) ASB2012-11419	Rabbit Himalayan	3 ♂	96.4	0.5 g moistened with water; intact skin	Non irritant
Leuschner, 2009b (Helm) ASB2012-11421	Rabbit Himalayan	3 ♂	98.8	0.5 g moistened with water; intact skin	Non irritant
Leuschner, 2010 (Helm) ASB2012-11422	Rabbit Himalayan	3 ♂	97.3	0.5 g moistened with water; intact skin	Non irritant
You, 2009 (Helm) ASB2012-11423	Rabbit NZW	1 ♂, 2 ♀	96.4	0.5 g moistened with water; intact skin	Non irritant
Merkel, 2005 (Helm) ASB2012-11424	Rabbit, NZW	3 ♂	97.23	0.5 g moistened with water; intact skin	Slightly irritating
Canabrava Frossard de Faria, 2008 (Helm) ASB2012-11425	Rabbit, NZW	3 ♀	98.05	0.5 g moistened with water; intact skin	Non irritant
Doyle, 1996 (Syngenta) TOX2000-1985	Rabbit, NZW	6 ♀	95.6	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
Arcelin, 2007 (Syngenta) ASB2012-11426	Rabbit NZW	1 ♂, 2 ♀	96.1	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
Zelenak, 2011 (Syngenta) ASB2012-11427	Rabbit NZW	3 ♂	96.3	0.5 g moistened with water; intact skin	Slightly irritating

NZW = New Zealand White

Of these 11 studies, 9 were unequivocally negative. Also the remaining two studies do not suggest a need for classification. Merkel (2005, ASB2012-11424) as well as Zelenak (2011, ASB2012-11427) reported very slight erythema in one animal that had, in both studies, cleared within 24 hours.

Thus, when compared to CLP criteria, glyphosate should not be classified and labelled for skin irritation.

In humans, skin irritation was seldom reported (Bradberry et al., 2004, ASB2012-11576). Most likely, the few documented cases were due to co-formulants in glyphosate-containing herbicides. Taking the extensive world-wide use of such products into account, skin irritation by glyphosate is not of concern for humans.

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 ≥ 2.3 - ≤ 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.

4.4.2 Eye irritation

In 1999, glyphosate was classified by the former European Chemicals Bureau as an eye irritant (Xi) and labelled with the risk phrase R41 ("Risk of serious damage to eyes"). This decision was based on a German proposal because of several findings of either eye irritation or at least slight irritation in all of a total of six studies that had been reviewed for first evaluation by the EU.

In preparation of the new EU evaluation, a number of studies were submitted that had not been reviewed before at EU level and are compiled in Table 14.

Table 14: Eye irritation tests with glyphosate acid in rabbits that had not been previously reviewed for classification and labelling purposes

Reference; Study identification; owner	Strain, number of Animals	Purity	Amount applied	Effects / Result
Kuhn, 1996; TOX1999-881; Cheminova	NZW, 6 male, 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, reversible within 21 days Irritant
Talvioja, 2007; ASB2012-11428; Nufarm	NZW, 1 male, 2 females	95.1%	100 mg	Marked, early onset and transient ocular changes (Cornea opacity, conjunctival redness, chemosis), reversible within 10 days, no signs of corrosion or staining Irritant
Leuschner, 2009; ASB2012-11429; Helm	Himalayan, 3 males	96.4%	100 mg rinsed 1h post appl.	Slight signs of ocular changes, reversible within 7 days Non-irritant
Hideo, 1995; ASB2012-11430; Arysta	NZW. 12 females	97.56%	100 mg (pure)	6 females without eye irrigation: Cornea opacity: not reversible within 21 days (3/6 females); iris lesions: all females and reversible within 10 days; conjunctival redness & chemosis: all females and reversible within 16 days; 6 females with eye irrigation (30 sec. & 2 min. post application): reduced effects and faster recovery Irritant
Leuschner, 2009; ASB2012-11432; Helm	Himalayan 3 males	98.8%	100 mg rinsed 1h post appl.	Non-irritant
Leuschner, 2010; ASB2012-11433; Helm	Himalayan 3 males	97.3%	100 mg rinsed 1 h post appl.	Non-irritant
You, 2009; ASB2012-11434; Helm	NZW 2 males 1 female	96.4%	0.1 mL (93.2 mg)	Cornea opacity, iris lesions, conjunctival redness & chemosis reversible within 9 days Irritant
Merkel, 2005; ASB2012-11435; Helm	NZW 3 males	97.23%	0.1 mL (60 mg)	All animals: corneal opacitiy, iris lesions, conjunctival redness & chemosis, reversible within 10 days Irritant
Canabrava Frossard de Faria, 2008; ASB2012-11436; Helm	NZW 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 Irritant
Reagan & Laveglia, 1988; Z35395; Monsanto	NZW 6 animals, likely 3/sex	97.76%	100 mg	One rabbit died: considered not treatment related Corneal opacitiy, iritis, conjunctival redness, chemosis in 6/6 animals. Some effects not reversible within 21 days Irritant
Johnson, 1997; TOX2000-1986;	NZW 6 females	95.6%	100 mg	Corneal opacity, iritis,conjunctival redness and chemosis. All effects reversible within 8 days

Reference; Study identification; owner	Strain, number of Animals	Purity	Amount applied	Effects / Result
Syngenta				Moderately Irritant (according to Kay & Calandra)
Arcelin, 2007; ASB2012-11437; Syngenta	NZW 1male 2 females	96.1%	100 mg	Mild, early-onset and transient ocular changes (reversible within 7 days) Irritant
Tavaszi, 2011 ASB2012-11438; Syngenta	NZW 1 male	96.3%	Glyphosate technical 100 mg	Based on results in one animal, study was terminated at 24 h: corneal opacity & erosion; conjunctiva: redness, chemosis, discharge, few black points; oedema of the eyelids; positive fluorescein staining at 24 h Corrosive

In a total of 13 studies, eye irritation by glyphosate was observed in 9 of them and a further one even revealed corrosive properties. The studies themselves are reported in detail in the attached Volume 3 (B.6.2.5) of the RAR. In contrast, glyphosate proved negative for eye irritation in three studies (Leuschner, 2009, ASB2012-11429; Leuschner, 2009, ASB2012-11432; Leuschner, 2010, ASB2012-11433). However, in these studies, rinsing of the eyes was performed one hour after instillation. This is not in compliance to the current OECD Guideline 405 in which rinsing is scheduled after 24 hours. In many studies, there was no rinsing at all. Thus, it may be assumed that the different outcome was due to this methodological change and that testing in these three experiments by the same researcher was not that rigorous as in the other studies. In three further studies in which test material from the same company (even though of different purity) was applied in another laboratory, the outcome was positive (Merkel, 2005, ASB2012-11435; Canabrava Frossard de Faria, 2008, ASB2012-11436; You, 2009, ASB2012-11434).

In any case, the majority of tests clearly pointed to the risk of eye irritation by glyphosate. Accordingly, the need for classification for eye irritation was confirmed. If category 1 or 2 is more appropriate, depends on the severity and reversibility of effects. Criteria for allocation to category 1 are the following:

- Effects on cornea, iris or conjunctiva at least in one animal that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or
- A positive response score (mean following grading at 24, 48, and 72 hours after instillation) for corneal opacity ≥ 3 and/or iritis > 1.5 in at least 2 of 3 animals.

At least one of these criteria was met in the studies by Tavaszi (2011, ASB2012-11438), by Canabrava Frossard de Faria (2008, ASB2012-11436), by Merkel (2005, ASB2012-11435) and by Reagan and Laveglia (1988, Z35395) whereas the other positive studies would instead support classifying glyphosate in category 2.

Since evidence of strong eye irritation was obtained in several (even though not in all) studies, it is proposed to assign category 1.

Accordingly, the current classification "Eye irritation, Category 1" is confirmed. The signal word is "Danger" and the appropriate hazard statement is H318: "Causes serious eye damage".

At least transient eye irritation is a rather frequent symptom in humans following contact with herbicides containing glyphosate (e.g., Acquavella et al., 1999, TOX2002-699). These observations

might be due to glyphosate confirming the animal evidence but may be also caused or or enhanced by co-formulants such as POEA surfactants which exhibit a strong eye-irritating potential themselves (see Vol. 3, B.6.13.3).

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 / Result
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 o 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. Fulfils the criteria for category 2.
Arcelin (2007); (study considered acceptable by DS)	NZW rabbit, 1male, 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 ot 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.

4.4.3 Respiratory tract irritation

Respiratory tract irritation might be expected because of the eye irritating potential of glyphosate and, in fact, could have actually occurred occasionally in acute inhalation studies (e.g., Tornai, 1994, TOX9650144, see Table 12) but cannot be clearly distinguished from inhalation toxicity. In any case, it would have been confined to high concentrations. In the current CLP guidance, it is stated that evaluation, in the absence of validated animal tests, will be based primarily on human data.

In humans, there is no evidence for respiratory tract irritation by the active substance even though one must acknowledge that such an exposure will seldom occur. For formulations, Burger et al. (2009, ASB2013-11831) reported cases from Germany that might indicate respiratory irritation but, most likely, these findings were due to POEA surfactants.

On balance, there is no sufficient evidence to classify glyphosate for respiratory tract irritation. It should be taken into account that glyphosate is classified and labelled for eye irritation and, thus, irritating properties are already adequately covered.

4.5 Corrosivity

Physico-chemical properties of glyphosate do not suggest corrosive potential. In line with that, evidence of corrosivity coming from the animal studies was confined to a single eye irritation study (Tavaszi, 2011, ASB2012-11438) but was not confirmed in a great number of similar studies for this endpoint or in any of the dermal toxicity or skin irritation studies.

Apart perhaps from the manufacturing process, humans will be always exposed to formulations containing the active ingredient rather than to the pure active ingredient. There were no reports to date pointing to corrosive properties of such formulations, despite clear evidence for eye or mucosal irritation.

Thus, glyphosate should not be considered corrosive and the proposed classification and labelling for eye irritation is adequate and sufficient.

4.6 Sensitisation

4.6.1 Skin sensitisation

There is no animal study suggesting skin sensitisation by glyphosate (see Vol. 3, B.6.2.6). In Table 15, the available and acceptable or at least supplementary maximisation (Magnusson and Kligman) tests and local lymph node assays (LLNA) are listed since they are considered more rigorous and reliable than the Buehler test. It should be noted that Buehler tests with glyphosate were also consistently negative.

Table 15: Summary of skin sensitisation studies with glyphosate acid

Study	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Result
Snell, 1994 (Herbex) TOX9500250	Guinea pig, Dunkin Hartley	15 ♀	95	Induction: 1% w/v in arachis oil; challenge: 25% w/w or 50% w/w in arachis oil	MK	Not sensitising
Pore et al, 1993 (Luxan) TOX9650652	Guinea pig, English	48 (both sexes)	≥95	Intradermal induction: 5% in propylene glycol; topical: 50% in petrolatum	MK	Not sensitising
Walker, 1991 (Agrichem) TOX9551796	Guinea pig Dunkin Hartley	38 ♀	Not stated	Intradermal induction: 0.1% (w/v) in water; topical: 50% (w/v) in water; challenge: 25% (w/w) in water	MK	Not sensitising
Cuthbert & Jackson, 1989 (Cheminova) TOX9552343	Guinea pig, Dunkin Hartley	46 ♀	98.6	Induction: 10% in water; challenge: 25% in water	MK	Not sensitising
Talvioja, 2007 (Nufarm) ASB2012-11439	Guinea pig	20 ♀/test 10 ♀/control	95.1	Intradermal induction: 3% (w/v) in PEG-300; topical induction: 50% (w/v) in PEG-300; challenge: 25% (w/v) in PEG-300	MK	Not sensitising
Haferkorn, 2010 (Helm) ASB2012-11440	Guinea pig, Dunkin Hartley	15 ♀ (+ 20 for positive control)	96.4	Intradermal induction: 0.01% in water; topical induction: 50%; challenge: 25%	MK	Not sensitising
Hideo, 1995 (Arysta) ASB2012-11441	Guinea pig, Hartley	60 ♀	97.56	Intradermal induction: 5% (w/v) in paraffin oil, topical induction: 25% (w/v) in white petrolatum; challenge: 25% (w/w) in white petrolatum	MK	Not sensitising
Simon, 2009 (Exxel) ASB2012-11442	Guinea pig	15 ♂	96.66	Intradernal induction: 10% (w/w) in purified water; topical induction: 50% (w/w) in purified water; challenge: 15% (w/w) in purified water	MK	Not sensitising
Haferkorn, 2009 (Helm) ASB2012-11443	Guinea pig	15 ♂ (+ 20 for positive control)	98.8	Intradermal induction: 0.01% in water, topical induction: 50%; challenge: 50%	MK	Not sensitising
Haferkorn, 2010 (HAG) ASB2012-11444	Guinea pig	15 ♂ (+ 20 for positive control)	97.3	Intradermal induction: 0.5% in water; topical induction: 50%; challenge: 25%	MK	Not sensitising
Richeux, 2006	Guinea pig	20 ♀/test	95.7	Intradermal induction:	MK	Not sensitising

Study	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Result
(Nufarm) ASB2012-11448		10 ♀/control		0.195% (w/v) in isotonic saline; topical induction: 60% (w/v) in water; challenge: 60% (w/v) & 30% (w/v) in water		
Doyle, 1996 (Syngenta) TOX2000-1987	Guinea pig	20 ♀/test 10 ♀/control	95.6	Intradermal induction: 0.1% (w/v) in water; topical induction: 75% (w/v) in water; challenge: 75% (w/v) & 30% (w/v) in water	MK	Not sensitising
Betts, 2007 (Syngenta) ASB2012-11449	Mouse, CBA	4 ♀/group	96.1	Glyphosate acid dose levels: 0, 10, 25, 45 (% w/v) Hexylcinnamaldehyde (positive control) demonstrated sensitivity of study	LLNA	Not sensitising
Török-Batho, 2011 (Syngenta) ASB2012-11450	Mouse, CBA	4 ♀/group	96.3	Glyphosate acid dose levels: 0, 10, 25, 50 (% w/v) Hexylcinnamaldehyde (positive control) demonstrated sensitivity of study	LLNA	Not sensitising

MK = Magnusson Kligman Maximisation Test
LLNA = Local Lymph Node Assay

Thus, there is unequivocal evidence that glyphosate did not produce skin sensitisation in laboratory animals. Classification and labelling are not needed. To date, there are no reports on skin sensitisation by glyphosate or its formulations in humans.

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, **no classification for skin sensitisation** is justified according to the CLP criteria.

4.6.2 Respiratory sensitisation

An appropriate animal model is not available. There is no evidence of respiratory sensitisation in humans by contact with formulations containing glyphosate.

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.

4.7 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.7.1 Non-human information

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. However, other study types, e.g. for reproductive or developmental toxicity, may also provide relevant information (see Guidance on the Application of the CLP Criteria, Version 4.1 – June 2015, 3.9.2.1.2. Identification of non-human data) and may possibly support a need for classification. The latter case is applicable to glyphosate but a comprehensive picture shall be given. Therefore, in this sub-section, the available short-term toxicity studies with glyphosate are reported first. Thereafter, non-cancer effects in long-term studies are considered. In the third part, maternal toxicity in developmental studies in rabbits is addressed since the new proposal for classification is based on mortality occurring in this animal model.

Short-term studies

A multitude of oral short-term studies with glyphosate was conducted mainly in rats and dogs. In addition, a small number of studies were performed in mice by the oral route or in rats and rabbits by dermal application.

Glyphosate was administered in few subacute studies (duration 14 or 28 days) by the oral route to rats and dogs. Toxicity upon dietary administration to rats was very low with only minor effects such as soft faeces or alterations in some haematological and clinical chemistry parameters at high dose levels (Suresh, 1991a-c, TOX9551095, Z102035, Z102043). The lowest NOAEL of 50 mg/kg bw/day as established by Atkinson et al. (1989, TOX9552351) was mainly based on a higher incidence of nephrocalcinosis in females at 250 mg/kg bw/day and above. However, this finding was not confirmed in a subsequent 90-day study employing more animals that was performed in the same laboratory and rat strain at much higher dose levels (Perry et al., 1991, TOX9552364). Therefore, and since there were no histopathological renal findings in any other short-term study with glyphosate in rats, nephrocalcinosis cannot be attributed to glyphosate administration. In dogs, there were no treatment-related findings observed up to 1000 mg/kg bw/day (Gobordhun and Oshodi, 1989, TOX9552352).

In both Sprague-Dawley (Heath et al., 1993, TOX9552367) and Wistar-derived rats (Pinto, 1996, ASB2012-11461) as well as in NZW rabbits (Johnson, 1982, TOX9552366; Tornai, 1994, TOX9650151), no signs of systemic toxicity became evident following repeated application of glyphosate to the skin over a period of 3 or 4 weeks up to the highest tested dose levels of 1000 mg/kg bw/day in the rat and 5000 mg/kg bw/day in the rabbit. However, weak dermal irritation was observed at these high dose levels in both species.

On balance, the subacute studies do not support a classification for STOT RE.

Subchronic studies (90 days or longer) with glyphosate were conducted by the oral route only.

The available studies in rats that are considered acceptable according to today's standards are summarised in Table 16. Taken together, all these studies have demonstrated low toxicity of glyphosate in different rat strains upon repeated oral administration. Soft stools and diarrhoea, together with occasionally reduced body weight gain, might suggest some irritation of the gastrointestinal tract at high dose levels that is not unexpected for a compound of acidic properties and known irritancy at least to the eyes. In the same studies, blood (Parker, 1993, TOX9650149) or haemoglobin (Coles et al., 1996, ASB2012-11451) were observed in urine at high dose levels. A decrease in urine pH was quite frequently noted.

These findings may be assumed to result from physico-chemical properties of glyphosate but this does not necessarily mean that they were not adverse. The same holds true for parotid salivary gland findings reported by Perry et al. (1991, TOX9552364). Histological alterations comprised deep basophilic staining and enlargement of cytoplasm at all dose levels including very few control animals but were clearly more pronounced with regard to incidence and severity at the top dose level in males and females. They were not accompanied by organ weight changes neither of the parotid nor of the sublingual or submaxillary glands. In the latter two glands, no histopathological changes were noted. The absence of indications for such changes in other studies may be explained by the fact that different or no glands had been examined. Parker (1993, TOX9650149) reported swelling and reddening of sublingual salivary glands in a few animals but no dose response became apparent and histological examination did not reveal any noteworthy findings. Salivary glands were not weighed. Eadie (1989, TOX9551821) and Suresh (1992, TOX9551096) did not report pathological changes in the salivary glands (not further specified). Stout and Johnson (1987, TOX9552362) examined the submaxillary gland only but did not detect any pathological changes. In the more recent studies by Botham (1996, TOX2000-1990) and Coles et al. (1996, ASB2012-11451), salivary glands were reported to be taken but were apparently not weighed or examined histologically. Kinoshita (1995, ASB2012-11452) performed histopathology of the sublingual and submaxillary glands without any noteworthy findings observed but left the parotid gland out of the investigation. Chan and Mahler (1992, TOX9551954), however, published a study in F344 rats in which they reported basophilic changes and hypertrophy of acinar cells in the submaxillary and, more pronounced, in the parotid salivary glands at all dose levels (ranging from 3125 to 50000 ppm). Severity of these findings were clearly related to dose and, based on severity, the NOAEL was set at 6250 ppm, equal to about 400 mg/kg bw/day (JMPR, 2004, ASB2008-6266). These findings directly supported the observations by Perry et al. (1991, TOX9552364).

Alterations in clinical chemistry parameters in the majority of experiments, most often a higher activity of alkaline phosphatase, suggested a weak effect on the liver.

Two studies (Kinoshita, 1995, ASB2012-11452; Coles et al., 1996, ASB2012-11451) identified the caecum as an additional target organ because of certain findings (distention, elevated weight of this part of the intestines and its contents, mucosal atrophy) that had not been noticed before. Even if a specific vulnerability of Sprague-Dawley rats would be assumed, it is difficult to explain why such changes were not observed previously at higher dose levels by Stout and Johnson (1987, TOX9552362), Perry et al. (1991, TOX9552364) or Parker (1993, TOX9650149). One might expect that at least caecal distention would have been observed and reported if it had occurred.

Table 16: Oral subchronic studies in rats

Reference; Study identification; Batch, purity; Owner	Strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Main effects
Botham, 1996; TOX2000-1990; P15, 97.4%; Syngenta	Wistar-derived (Alpk:APfSD), 90 d, feeding	0, 1000, 5000, 20000 ppm	414 mg/kg bw/d (5000 ppm)	1612 mg/kg bw/d (20000 ppm)	Bw gain↓ in m; alterations in some clinical chemistry parameters, in particular AP/ALAT activity↑, urine pH↓
Coles et al., 1996; ASB2012-11451; H95D 161 A, 95.3%; Nufarm	Sprague-Dawley (CD), 90 d, feeding	0, 1000, 10000, 50000 ppm	79 mg/kg bw/d (1000 ppm)	730 mg/kg bw/d (10000 ppm)	Soft faeces, diarrhea; bw gain, food consumption, food efficiency↓ and hemoglobin in urine at top dose level, urine pH↓; alterations in some clinical chemistry parameters, in particular AP activity↑ and Ca↓ at mid and high dose levels; caecum: distention (top dose groups) and mucosal atrophy (at the two upper dose levels)
Kinoshita, 1995; ASB2012-11452; Batches: 940908, 95.7%; 941209, 95%; T-941209; 97.6%; Arysta	Sprague-Dawley (Crj: CD), 90 d, feeding	0, 3000, 10000, 30000 ppm	168 mg/kg bw/d (3000 ppm)	569 mg/kg bw/d (10000 ppm)	Bw gain↓ in m; alterations in some clinical chemistry parameters, in particular AP activity↑, urine pH↓; caecum: distention and wt (with contents)↑
Perry et al., 1991; TOX9552364; Batch 206-JaK-25-1, 98.6%; Cheminova	Sprague-Dawley, 90 d, feeding	0-20-300-1000 mg/kg bw/d (dietary levels weekly adjusted)	300 mg/kg bw/d	1000 mg/kg bw/d	Bw gain↓ in m, urine pH↓ and some changes in clinical chemistry parameters in f ; m/f: cellular alterations in parotid salivary glands
Parker, 1993; TOX9650149; Lot 46540992, purity not given; Alkaloida#	Sprague-Dawley, 90 d, feeding	0, 2000, 6000, 20000 ppm	371 mg/kg bw/d (6000 ppm)	1262 mg/kg bw/d (20000 ppm)	Diarrhea in m/f; blood in urine; organ wt changes without pathological findings
Suresh, 1992; TOX9551096; Batch 60, 96.8%; ADAMA#	Wistar, 90 d (+28 d recovery, hig dose), feeding	0, 200, 2000, 20000 ppm (+20000 ppm for recovery) group)	147 mg/kg bw/d (2000 ppm)	1359 mg/kg bw/d (20000 ppm)	Bw gain↓ in f; AP activity↑ in m, glucose↑ in f
Eadie, 1989; TOX9551821; Batch L16566, 97.1%; Barclay	Sprague-Dawley (CD), 90-92 d (+35 d recovery for additional control and top	0, 2000, 3000, 5000, 7500 ppm (+ 7500 ppm for recovery)	7500 ppm (375 mg/kg bw/d assumed, mean dietary intake not caclculated)	>7500 ppm	No effects up to highest dose

Reference; Study identification; Batch, purity; Owner	Strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Main effects
	dose groups)				
Stout and Johnson, 1987; TOX9552362; Lot XLG 161, 95.2%; Monsanto	Sprague-Dawley, 90 d, feeding	0, 1000, 5000, 20000 ppm	1267 mg/kg bw/d (20000 ppm)	>1267 mg/kg bw/d (20000 ppm)	No effects up to highest dose

[#] supplementary study

It should be explained here that the "main effects" were statistically significant if body weight and organ weights were affected and haematological or clinical chemistry parameters altered. Clinical signs and histological lesions were also reported when occurring in a higher number of animals as in the control group but were not always subject to statistical evaluation or did not gain statistical significance in all cases. Not all of the mentioned findings were observed necessarily at the LOAEL but sometimes only at higher dose levels. This table (as well as Tables 17 and 18 below) is more intended to give an impression of the effect pattern. In any case, statistical significance was taken into account when the NOAELs/LOAELs in the individual studies were established.

In the dog, short-term toxicity (if compared to the life-expectancy of the species) of glyphosate was investigated in a number of studies with oral administration, either via capsules or in the diet. The valid subchronic dog studies (90 days or 1 year) are summarised in Table 17.

On the whole, the results have shown that the dog is of similar sensitivity as the rat when the NOAELs/LOAELs are considered. There is limited evidence coming from one study that high dose effects may be more severe than in rats or mice but these observations appear somehow inconsistent among the studies.

In the most recent 90-day study by Gaou (2007, ASB2012-11454), severe signs of toxicity were noted in the high dose groups receiving 1000 mg/kg bw/day. The test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) and body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11. Treatment-related histopathological changes in surviving animals consisted of an increased number of adipocytes in the sternal bone marrow in both sexes, as well as prostate and uterine atrophy and other, more infrequent changes in various organs. It is clear that the Maximum Tolerable Dose (MTD) was by far exceeded. In contrast, in the study by Gobordhun (1991, TOX9552384), the same high dose of 1000 mg/kg bw/day was administered also in capsules but for one year causing only minor effects. There is no explanation for this apparent difference although it is known from long-term studies in rats and mice that high-dose effects of glyphosate may differ considerably. A lower purity (and other source) of the test material applied by Gaou (2007, ASB2012-11454) might be relevant.

In 90-day or one-year studies with dietary administration, very few findings were obtained suggesting that glyphosate was better tolerated when administered via the diet than in capsules.

Prakash (1999, ASB2012-11455) reported an initial decline in food consumption and body weight gain but normalisation to control levels was quickly achieved. The only clinical chemistry alteration that was likely related to treatment, i.e., a higher bilirubin concentration, was not accompanied by any pathological change. Thus, these effects were not regarded as adverse.

In the study by Hodge (1996, TOX2000-1991), weak toxic effects were noted at the exaggerated top

dose of 50000 ppm, including a decrease in body weight gain and some evidence of liver toxicity. The next lower dietary level of 10000 ppm (approx. 320 mg/kg bw/day) was considered the NOAEL. In line with that, Yoshida (1996, ASB2012-11456) did not find any effects (apart from a reduction in urine pH due to acidic properties of the test substance) in a study in which even higher dietary dose levels of up to 40000 ppm were employed.

Table 17: Subchronic oral studies with glyphosate in dogs

Reference; Study identification; Batch, purity; Owner	**Breed, duration, route**	**Dose levels**	**NOAEL**	**LOAEL**	**Targets / Main effects**
Gaou, 2007; ASB2012-11454; H05H016A, 95.7%; Nufarm	Beagle, 13 week, oral capsules	0, 30, 300, 1000 mg/kg bw/d	300 mg/kg bw/d	1000 mg/kg bw/d	Clinical signs (liquid/soft faeces, dehydration, vomi-ting) making termination of high dose groups after 11 wk necessary; bw/bw gain and food consumption↓; clinical chemistry and urine parameters altered; prostate aund uterus atrophy; histological lesions in many organs (such as kidney liver, bone marrow) related to moribund state
Prakash, 1999; ASB2012-11455; Lots 01/12/1997 and 01/06/1997, >95% both; ADAMA	Beagle, 90 d, dietary	0, 200, 2000, 10000 ppm (equal to 5.2/5.4; 54.2/52.8, 252.4/252.7 mg/kg bw/d in m/f)	252 mg/kg bw/d	>252 mg/kg bw/d	No adverse effects up to highest dose level
Yoshida, 1996; ASB2012-11456); T940308, 94.61%; Arysta	Beagle, 13 week, dietary	0, 1600, 8000, 40000 ppm (approx. 40, 198/201, 1014/1015 mg/kg bw/d in m/f)	1014 mg/kg bw/d	>1014 mg/kg bw/d	Decrease in urine pH in high dose females not regarded as adverse; no further effects
Hodge, 1996; TOX2000-1991; Lots D4490/1, P18, 99.1%; Syngenta	Beagle, 90 d, dietary	0, 2000, 10000, 50000 ppm (68/68, 323/334, 1680/1750 mg/kg bw/d in m/f)	323 mg/kg bw/d	1680 mg/kg bw/d	Bw gain↓; alterations in some clinical chemistry parameters (calcium, albumin↓ in m, AP↑ in f); liver wt↑
Haag, 2008; ASB2012-11457; H05H016A, 95.7%; Nufarm	Beagle, 52 wk, capsules	0, 30, 125, 500 mg/kg bw/d	500 mg/kg bw/d	>500 mg/kg bw/d	No adverse effects, calcium↓ in high dose m
Nakashima, 1997; ASB2012-11458; T-950380, 94.61%; Arysta	Beagle, 12 month, dietary	0, 1600, 8000, 50000 ppm (34/37, 182/184, 1203/1259 mg/kg bw/d in m/f)	182 mg/kg bw/d	1203 mg/kg bw/d	Bw gain↓, loose stool, alterations in some hematological and clinical chemistry parameters
Brammer, 1996; TOX2000-1992; P24, 95.6%; Syngenta	Beagle, at least one year, dietary	0, 3000, 15000, 30000 ppm (*ca* 91, 440/447, 907/926 mg/kg	447 mg/kg bw/d	926 mg/kg bw/d	Bw gain↓ in f

Reference; Study identification; Batch, purity; Owner	Breed, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
		bw/d in m/f)			
Gobordhun, 1991; TOX9552384; 206-JaK-25-1, 98.6%; 206-JaK-95-5, 99.5%; 229-JaK-5-1, 98.9%; Cheminova (/Monsanto)	Beagle, 52 week, oral capsules	0, 30, 300, 1000 mg/kg bw/d	300 mg/kg bw/d	1000 mg/kg bw/d	Soft/loose/liquid stool, evidence of lower bw gain (not attending statistical significance)

Again, statistical significance was achieved for most effects on body weight, liver weight and laboratory parameters, if not the contrary is indicated. Clinical signs and histological findings were considered on the basis of individual animals affected. In general, statistical considerations are less important for a study with low numbers of individuals per dose level.

Toxicity of glyphosate to mice was investigated in a small number of subchronic studies. The NOAEL in the most recent valid 90-day study was 1221 mg/kg bw/day (Kuwahara, 1995, ASB2012-11453). A very high dose of approx. 6300 mg/kg bw/day caused a reduction in body weight gain, food consumption and efficiency and alterations in some haematological and clinical chemistry parameters with the latter findings pointing to liver toxicity. Gross necropsy revealed caecum distention that was supported by a higher organ weight but not accompanied by histological lesions. Cystitis of urinary bladder became histologically apparent in some high dose males. Urinary pH (most likely due to acidic properties of the test substance) was noted in all treated male groups. In a previous study (Perry et al., 1991, TOX9552363), no effects were observed up to the highest dose level of 4500 mg/kg bw/day. While these two studies would suggest a lower toxicity in mice than in the rat, a published study from the U.S. NTP (Chan and Mahler, 1992, TOX9551954) provided a lower NOAEL of about 500 mg/kg bw/day in another strain, based on histological changes in the parotid gland at about 1065 mg/kg bw/day and above. The findings comprised increased basophilia but also enlarged cells and acini with relative reduction in the number of acinar ducts. In the studies by Kuwahara (1995, ASB2012-11453) and Perry et al., (1991, TOX9552363), no effects on sublingual or submaxillary glands were noted but the parotid gland was not examined although it is obviously more sensitive to histological changes caused by glyphosate. Taking the salivary gland findings into account, toxicity of glyphosate acid in the mouse appears similar to that in the rat.

Long-term studies

Chronic toxicity, i.e., occurrence of non-neoplastic effects in studies of longer duration, might be also relevant for a STOT RE classification. With glyphosate, a large number of long-term studies have been performed in rats and mice. In a one-year feeding study for chronic toxicity in Wistar-derived rats, Milburn (1996, TOX2000-1998) observed effects on body weight, food consumption and food efficiency as well as an increase in alkaline phosphatase activity and focal basophilia of acinar cells of parotid salivary gland. Unfortunately, the weight of the parotid gland was not determined. Effects occurred from a dietary dose of 8000 ppm (corresponding to 560 mg/kg bw/day in male rats and to 671 mg/kg bw/day in females) onwards with the NOAEL being the next lower dose of 2000 ppm (equal to 141 or 167 mg/kg bw/day).

The long-term (2 years) combined chronic toxicity and carcinogenicity studies in rats and the

carcinogenicity studies in mice (18 months or 2 years) are reported in the section on carcinogenicity. Here, it is sufficient to state that an overall NOAEL for the rat studies in the magnitude of 100 mg/kg bw/day may be derived whereas first effects were seen in the range of 300-400 mg/kg bw/day in at least three studies (Stout and Ruecker, 1990, TOX9300244; Atkinson et al., 1993, TOX9750499; Enomoto, 1997, ASB2012-11484) whereas the LOAELs were much higher in the remaining studies. High-dose effects differed considerably among the studies (see Table 25 below). In mice, the overall NOAEL for long-term toxicity in the mouse can be set at 150 mg/kg bw/day, based on the studies by Sugimoto (1997, ASB2012-11493), Kumar (2001, ASB2012-11491) and Knezevich and Hogan (1983, TOX9552381). The overall LOAEL was around 800 mg/kg bw/day. The lowest doses at which effects were observed were 787 mg/kg bw/day in females in the study by Sugimoto (1997, ASB2012-11493) and 814 mg/kg bw/day in males in the study by Knezevich and Hogan (1983, TOX9552381). For details, see Table 30 in the carcinogenicity section. As in rats, the nature of high dose effects in mice was different in the various studies, depending on laboratory, strain, dose selection and, perhaps, purity and impurities profiles of the applied test material.

Reproductive and developmental studies

A large number of multi-generation studies on rats and of developmental (teratogenicity) studies on rats and rabbits is available. These studies are addressed in section 4.10. For possible classification for STOT RE, only the parental or maternal toxicity in these studies might be of interest and concern. In the rat, treatment-related findings were consistently confined to very high doses. This is shown by NOAELs for parental toxicity in the two-generation studies that range from 197 to approximately 700 mg/kg bw/day. The lowest dose levels at which adverse effects occurred ranged between 668 and > 1000 mg/kg bw/day (see Table 46). In the developmental studies, the lowest NOAEL for maternal toxicity was 300 mg/kg bw/day but, in most studies, no effects were seen up to the limit dose of 1000 mg/kg bw/day (see Table 47).

In contrast, the pregnant rabbit turned out to be the most vulnerable animal model when glyphosate was tested. An “overall” maternal NOAEL of 50 mg/kg bw/day was established in a total of 7 developmental studies, taking into account dose spacing. It was based on mortality, abortions, reductions in body weight (gain) and food consumption and gastro-intestinal clinical signs such as loose stool or diarrhoea. The LOAEL is 100 mg/kg bw/day. At this dose level, there were maternal deaths in the study by Suresh (1993, TOX9551106). An overview on maternal deaths and non-lethal effects in the rabbit studies is provided in Table 18. It should be emphasised that the studies by Bhide and Patil (1989, TOX9551960) and by Suresh (1993, TOX9551106) are only supplementary due to inferior quality but for the endpoint under consideration (maternal toxicity and mortality) they may be taken into consideration. Only those fatalities are listed in the table that can be attributed to treatment. Additional cases are indicated by asterisks. Some of the maternal deaths (the single mortalities in the studies by Hojo and by Brooker, 3 out of 8 at the high dose level in the study by Suresh and one in the study by Coles and Doleman) occurred after cessation of treatment. Nonetheless, it seems reasonable to consider them treatment-related.

Table 18: Maternal mortality and toxicity in the developmental studies with glyphosate in rabbits (all by oral gavage)

Reference; Study identification; Batch, purity; Owner	Strain, duration of treatment	Dose levels	Number of does per group	Premature deaths and dose level(s) at which they occurred	Further maternal effects	Maternal NOAEL / LOAEL (mg/kg bw/d)
Tasker et al., 1980; TOX9552390; Lot XHJ-64, 98.7%; Monsanto	Dutch Belted rabbit, d 6-27 p.c., gavage	0, 75, 175, 350 mg/kg bw/d	16	1 at 175, 7 at 350 mg/kg bw/d	Soft stool, diarrhea	75 / 175
Bhide & Patil, 1989; TOX9551960; Lot 38, 95%; Barclay, Luxan	NZW rabbit, d 6-18 p.c., gavage	0, 125, 250, 500 mg/kg bw/d	15	None	Food consumption, bw↓, abortion	250 / 500
Brooker et al., 1991; TOX9552391; 206-Jak-25-1, 98.6%; Cheminova	NZW rabbit, d 7-19 p.c., gavage	0, 50, 150, 450 mg/kg bw/d	16 – 20	1 at 450 mg/kg bw/d	Soft/liquid stool, food consumption and bw gain ↓, abortion	50 / 150
Suresh et al., 1993; TOX9551106; Batch 60, 96.8%; ADAMA	NZW rabbit, d 6-18 p.c., gavage	0, 20, 100, 500 mg/kg bw/d	15 – 17 in treated groups, 26 in control	4 at 100, 8 at 500 mg/kg bw/d**	Soft/liquid stool	20 / 100
Hojo, 1995, ASB2012-11498; T-041209, 97.56%; Arysta	Japanese White rabbits (Kbl:JW), d 6-18 p.c., gavage	0, 10, 100, 300 mg/kg bw/d	18	1 at 300 mg/kg bw/d	Loose stool, abortion	100 / 300
Coles & Doleman, 1996; ASB2012-11499; H95D161A, 95.3%; Nufarm	NZW rabbit, d 7-19 p.c., gavage	0, 50, 200, 400 mg/kg bw/d	18	2 at 400 mg/kg bw/d	Food consumption, bw gain ↓, scours	50 / 200
Moxon, 1996; TOX2000-2002; Y04704/034, 95.6%; Syngenta	NZW rabbit, d 8-20 p.c., gavage	0, 100, 175, 300 mg/kg bw/d	20	None***	Food consumption, bw gain ↓, diarrhea	100 / 175

*Five additional deaths (one in the control and mid dose group each and 3 at the top dose level were attributed to diseases such as pneumonia or gastroenteritis but not to treatment.
** Two deaths in the control group were due to misdosing and clearly not treatment-related.
***In fact, there were 1, 2, 2, and 2 intercurrent deaths in the four groups, mostly related to abortion. Since no dose response was seen, mortality and abortions were not considered treatment-related.

The majority of the maternal deaths did not reflect an acutely toxic effect since they occurred after some days of treatment at least or even around the end of the administration period. A few early deaths were confined to the study by Suresh (1993, TOX9551106) in which 3 does died on the first day of treatment. Two of these deaths were noted in the mid dose group but only one after administration of the high dose. If they were in fact due to acute oral toxicity of glyphosate to pregnant female rabbits, one would have expected a higher number to occur at the top dose level. In contrast, these early deaths rather suggest misgavaging even though this was not reported by the study author. The other four studies in which does died suggest a different time pattern of mortality supporting the

assumption of an effect of repeated administration. With regard to the individual studies, the days on which does died or were found dead are depicted in Table 19.

Table 19: Temporal occurrence of treatment-related maternal deaths in the developmental studies with glyphosate in rabbits

Reference; Study identification	Strain, duration of treatment	Dose levels	Day of first death with dose level	Days of further deaths with dose level
Tasker et al., 1980; TOX9552390	Dutch Belted rabbit, d 6-27 p.c.	0, 75, 175, 350 mg/kg bw/d	14 (350 mg/kg bw/d)	17, 18, 21 (350 mg/kg bw/d); 25 (175mg/kg bw/d)
Brooker et al., 1991; TOX9552391	NZW rabbit, d 7-19 p.c.	0, 50, 150, 450 mg/kg bw/d	20 * (450 mg/kg bw/d)	None
Suresh et al., 1993; TOX9551106	NZW rabbit, d 6-18 p.c.	0, 20, 100, 500 mg/kg bw/d	7 (2x 100 mg/kg bw/d; 1x 500 mg/kg bw/d)	11, 14, 15, 18, 19* (500 mg/kg bw/d) 9, 18 (100 mg/kg bw/d)
Hojo, 1995, ASB2012-11498	Japanese White rabbits (Kbl:JW), d 6-18 p.c.	0, 10, 100, 300 mg/kg bw/d	20* (300 mg/kg bw/d)	None
Coles & Doleman, 1996; ASB2012-11499	NZW rabbit, d 7-19 p.c.	0, 50, 200, 400 mg/kg bw/d	19 (400 mg/kg bw/d	20* (400 mg/kg bw/d

*mortality occurring after cessation of treatment

4.7.2 Human information

Not available.

4.7.3 Other relevant information

There are some publications of varying quality describing studies of different types and duration. These studies were performed with formulations and not with the active substance. Therefore this information is not considered for the classification and labelling proposal for glyphosate itself. However, this published information is reported in the attached RAR.

4.7.4 Summary and discussion

In short-term and chronic studies in rats, mice, and dogs, toxic effects of glyphosate were confined to rather high doses. The large differences in the NOAELs/LOAELs in the individual studies are due to dose spacing but it seems clear that in no species effects below 300 mg/kg bw/day should be anticipated. Even effects at higher dose levels are relatively minor in nature but may differ among the studies or the same endpoint and in the same species, depending on strain, laboratory and perhaps also test material (e.g., impurities). Compound–related findings comprised lower body weight gain, rather slight alterations in clinical chemistry and haematological parameters as well as a lower urine pH and clinical signs that indicate gastrointestinal irritation or disturbances. More pronounced toxicity was only seen in a single dog study with capsule administration at the high dose level of 1000 mg/kg bw/day.

Low toxicity of glyphosate upon repeated administration was confirmed in reproduction and developmental studies in rats. In contrast, the pregnant rabbit was much more vulnerable with a much lower maternal NOAEL of 50 mg/kg bw/day and an LOAEL of 100 mg/kg bw/day at which already mortality occurred in at least one study.

4.7.5 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Based on the nature and severity of toxic effects of glyphosate and the NOAELs and LOAELs for the different endpoints in the different species, it may be concluded that only maternal toxicity as observed in the developmental studies in rabbits is of concern with regard to classification as STOT RE. Accordingly, comparison with criteria should be confined to this endpoint and data.

4.7.6 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

The following criteria for classification for specific target organ toxicity – repeated exposure are given in CLP regulation:

CLP criteria
Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case): Rat: 28-day: ≤ 30 mg/kg bw/d 90-day: ≤ 10 mg/kg bw/d Category 2 (H373) Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be Harmful to human health following repeated exposure. Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case): Rat: 28-day: ≤ 300 mg/kg bw/d 90-day: ≤ 100 mg/kg bw/d

For an exposure period of shorter duration as is the case in a developmental study, at least the guidance value for the 28-day study should be considered. Even though the guidance values refer to studies in rats, there is no reason not to take into account effects that had occurred in the rabbit.

Based on the NOAEL of 50 mg/kg bw/day and the LOAEL of 100 mg/kg bw/day for maternal toxicity, category 2 seems most appropriate because these dose levels were clearly below the 28-day

guidance values for category 2 but higher than those that would qualify for category 1.

Since the proposal is based on mortality, no organ can be mentioned in brackets as it is recommended but not strictly required by the CLP regulation.

4.7.7 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

It is proposed to classify glyphosate as STOT RE, Category 2. The signal word is "Warning" and the appropriate hazard statement would be H373 (May cause damage to organs through prolonged or repeated exposure).

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 ≤ 10 mg/kg bw/d and STOT RE 2: 10 < C ≤ 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 ≤ 10 mg/kg bw/d and STOT RE 2: 10 < C ≤ 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 ≤ 2.5 mg/kg bw/d and STOT RE 2: 2.5 < C ≤ 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 LOEAL 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 *et al*., 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)	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

	Further deaths: Day 17, 18, 21 (350 mg/kg bw/d); 22, 25 (175 mg/kg bw/d); 26 (75 mg/kg bw/d) Abortions; 2 (GD 22), 1 (GD 27), 1 (GD 23) were sacrificed after abortion at 0, 175 and 350 mg/kg bw/d		
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 *et al*., 1991; 98.6%, 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.	Found dead: 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. Two other deaths were unrelated to the treatment (broken hindleg at 450 mg/kg bw/d and congenital abnormality in control group) Abortions; 1 at 50mg/kg bw/day (whole litter). 1 at 150 mg/kg bw/day (aborted 1 of 9 foetuses, remaining litter values are included in assessment).	Soft/liquid stool (2, 5, 13 animals at 50, 150 and 450 mg/kg bw/d) (dose-related increase). 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. A slight reduction in bw gain from GD 11 to termination at 150 and 450 mg/kg bw/d.	50 / 150 Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750
Suresh *et al*., 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.	Found dead: 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. First death; Day 7 (2x 100 mg/kg bw/d; 1x 500 mg/kg bw/d) Further deaths: Day 9, 18 (100 mg/kg bw/d) 11, 14, 15, 18, 19 (500 mg/kg bw/d) Abortions: No information regarding abortions.	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.	20 / 100 Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750
Hojo, 1995; 97.56%, Japanese White rabbit (Kbl:JW),	Found dead: 1 dead at 300 mg/kg bw/d (no clinical signs), day 20	4 animals showed loose stool in the high dose group. Loose stool were also seen in two control animals and in one	100 / 300

GD 6-18, gavage, 0, 10, 100, 300 mg/kg bw/d, 18 female rabbits per group Study considered acceptable in RAR.	<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).	animal in the low dose group. No significant effect on food consumption and body weight.	Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750
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.	<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) <u>Abortions;</u> The animal killed in extremis day 20 showed signs of abortion.	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.	50 / 200 Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750
Moxon, 1996; 95.6%, NZW rabbit, GD 8-20, gavage, 0, 100, 175, 300 mg/kg bw/d, 20 female rabbits per group Study considered acceptable in RAR.	Abortions; 1 in control (day 30), 2 at 100 mg/kg bw/d (day 19 and 25), 1 at 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.	Diarrhoea, ↓food consumtion accompanied by a stat. sign. ↓ bw gain in high dose group from GD 17-26	100 / 175 Corrected guidance values; STOT RE 1: ~75 STOT RE 2: ~750

* 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 consumtion 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 decendents 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.

4.8 Germ cell mutagenicity (Mutagenicity)

4.8.1 Non-human information

In a narrow sense, this hazard classification relates to the ability of a substance to induce heritable mutations, i.e., in germ cells. As compared to the extremely large database on toxicity and also genotoxicity of glyphosate, the available information to directly address this endpoint is scarce. Glyphosate has been shown to be devoid of mutagenic activity in dominant lethal assays when applied as a single oral dose of up to 2000 mg/kg bw to CD-1 mice (Wrenn et al., 1980, TOX9552377) and of up to 5000 mg/kg bw to Wistar rats (Suresh, 1992, TOX9551102).

Thus, as for most substances, evaluation of a mutagenic potential must mainly rely on studies that address mutagenicity and genotoxicity of the active substance glyphosate in somatic cells. A broad spectrum of mutagenicity and genotoxicity tests in vitro and in vivo is available for glyphosate and glyphosate based formulations which is summarised in the following sub-sections with regard to gene mutations in bacteria and somatic cells, chromosome aberrations *in vitro* and in intact animals and direct interaction with the DNA (comprising, *e.g.*, UDS or Comet assays).

The DS is aware that, in addition to the studies with glyphosate, a large number of published studies with formulations containing glyphosate are available which were tested for different mutagenicity and genotoxicity endpoints in a variety of *in vitro* and *in vivo* mammalian and non-mammalian test systems. A part of these studies revealed positive or at least equivocal results in particular when testing was performed in non-standard systems and when so-called “indicator tests” were employed. It is likely that such results were rather due to co-formulants than to glyphosate. Therefore, they cannot be taken into account for classification of glyphosate for mutagenicity. Furthermore, against the background of an extremely large database using standard test systems (bacteria, mammalian cells and mammals), data obtained in non-standard test systems (e.g. plant, insect, worm, fish etc.) was not considered for classification of health related endpoints even if performed with the active ingredient. Therefore, all this information is not provided in this CLH report but may be found in the attached RAR.

Table 20: Summary of germ cell mutagenicity tests in mammals, *in vivo*

Reference	**Species, test, tissue**	**Test substance, purity, application route, dose levels, mating period**	**Results by authors**	**GLP, Test guideline**	**Result details**	**Comments**
Wrenn et al. 1980, TOX9552377	Mouse, Dominant lethal test	Glyphosate, 98.7 % oral, 1x 0, 200, 800 or 2000 mg/kg bw 8 successive one-week mating periods (1 male/2 females)	Negative	GLP, no reference to TG	No increase in post-implantation loss in treated groups. PosControl: stat. significant increase in post-implantation loss.	Only 10 males per group. Post-implantation loss evaluated after mating of non-treated females with glyphosate-treated male mice.
Suresh, 1992, TOX9551102	Rat, Dominant lethal test	Glyphosate, 96.8 % oral, 1x 0, 200, 800 or 2000 mg/kg bw 10 successive one-week mating periods (1 male/1 female)	Negative	GLP, OECD 478 (1984)	No increase in post-implantation loss in treated groups. PosControl: stat. significant increase in post-implantation loss.	30 males per group (Control: 10 males, PosControl: 2 x 5 males). Post-implantation loss evaluated after mating of non-treated females with glyphosate-treated male mice.

4.8.1.1 *In vitro* data

The ability of glyphosate to cause gene/point mutations in bacteria was investigated in numerous studies by means of the reverse mutations ("Ames") test giving consistently negative results. The available studies were all run with and without metabolic activation, using liver S9 mix to mimic in vivo liver metabolism. The available valid studies, 16 in total, are compiled in Table 21, along with a Rec assay in *Bacillus subtilis* for investigations of a possible interaction with bacterial DNA.

Table 21: Summary of *in vitro* mutagenicity and genotoxicity tests with glyphosate acid in bacteria

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels; purity; metabolic activation	Results
Jensen, 1991; TOX9552371; Cheminova	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537	- S9: 160 – 2500 µg/plate; + S9: 310 – 5000 (plate-incorporation and pre-incubation test); 98.6%	Negative
Shirasu et al., 1978; TOX9552368; Monsanto	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537, 1538 and *E. coli* WP2 hcr	10 – 5000 µg/plate (plate-incorporation assay); 98.4%; +/- S9	Negative (supplementary study)
Akanuma, 1995a; ASB2012-11462: Arysta	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	156-5000 µg/plate (pre-incubation test); 95.68%; +/- S9	Negative (supplementary study)
Sokolowski, 2007a; ASB2012-11463; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation), 33 – 5000 µg/plate (pre-incubation test); 95.1%; +/- S9	Negative
Sokolowski, 2007b; ASB2012-11464; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test); 97.7%; +/- S9	Negative
Sokolowski, 2007c; ASB2012-11465; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test); 95.0%; +/- S9	Negative
Riberri do Val, 2007; ASB2012-11466; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	648 – 5000 µg/plate (plate–incorporation); 98.01%; +/- S9	Negative (supplementary study)
Flügge, 2009a; ASB2012-11468; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate-incorporation and pre-incubation test); 98.8%; +/- S9	Negative
Flügge, 2010; ASB2012-11469; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate incorporation and pre-incubation test); 96.4%; +/- S9	Negative
Sokolowski, 2010; ASB2012-11470; Helm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate incorporation and pre-incubation test); 97.16% technical a.i. containing 0.63% glyphosine; +/- S9	Negative
Wallner, 2010;	Ames test	*S. typhimurium* TA 98,	31.6 – 5000 µg/plate (plate	Negative

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels; purity; metabolic activation	Results
ASB2012-11471; Helm		100, 102, 1535, 1537	incorporation and pre-incubation test); 98.2%; +/- S9	
Thompson, 1996; ASB2012-11472; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	0 – 5000 µg/plate (plate–incorporation); 95.3%; +/- S9	Negative (supplementary study)
Callander, 1996; ASB2012-11473; Syngenta	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2P *uvrA* and WP2P	100 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 95.6%; +/- S9 (for pre-incubation test only with S9 mix)	Negative
Sokolowski, 2009; ASB2012-11474; Syngenta	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2 *uvrA* pKM 101and WP2 pKM 101	3 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 96.3%; +/- S9	Negative
Schreib, 2012; ASB2014-9133; Industria Afrasa	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	10 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 97%; +/- S9	Negative
Thompson, 2014; ASB2014-9148; Albaugh	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2 *uvrA*	1.5 or 5 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 85.79%; +/- S9	Negative
Akanuma, 1995b; ASB2012-11477; Arysta	Rec assay	*B. subtilis* strains H17 and M45 (+/- S9)	+/- S9 : 7.5 – 240 µg/disk; Lot 940908-1; 95.68%	Negative (supplementary study)

Absence of mutagenicity *in vitro* was further confirmed in a number of studies for point (gene) mutations in mammalian cells, i.e., in two mouse lymphoma assays (Jensen, 1991, TOX9552372; Clay, 1996, TOX2000-1994) and an HPRT test (Li, 1983, TOX9552369). No evidence of clastogenicity was obtained in four valid in vitro studies in human lymphocytes (Van de Waart, 1995, TOX9651525; Fox, 1998, TOX2000-1995) or Chinese hamster lung cells (Kyomu, 1995, ASB2012-11475; Wright, 1996, ASB2012-11476). The conclusion that glyphosate was not clastogenic in vitro was also supported by the negative outcome of the two mouse lymphoma assays (Jensen, 1991, TOX9552372; Clay, 1996, TOX2000-1994). In an UDS assay in rat hepatocytes (Rossberger, 1994, TOX9400697), there was no impact on DNA damage and repair.

Other studies in mammalian cells, in contrast, revealed positive results or contradictory findings. On one hand, Lioi et al. (1998a, ASB2013-9836; 1998b, ASB2013-9837) reported higher rates of SCE and chromosome aberrations when glyphosate (purity ≥98%) was tested in human and bovine lymphocytes in vitro at the maximum concentrations of 51 or 170 µM. Bolognesi et al. (1997, Z59299) found evidence of increased sister chromatid exchange (SCE) in human lymphocytes for 99.9% pure glyphosate at dose levels of 1 mg/mL up to 6 mg/mL. Mladinic et al. (2009a, ASB2012-11907) reported an increase in micronucleus formation in human lymphocytes at the highest and already cytotoxic concentration of 580 µg/mL (approx. 3.43 mM) when S9 mix had been added. Koller et al. (2012, ASB2014-7618) observed an increase in micronucleus frequency in human cells of buccal origin (carcinoma cell line TR146) after treatment with an aqueous solution of 95% technical grade glyphosate for 20 minutes. For this investigation, the cytokinesis-block micronucleus cytome assay was employed. A significant (Chi-square test with Yate's correction, $p \leq 0.001$) and dose-related increase was seen at the upper concentrations of 15 and 20 µg/mL. On the other hand, chromosome aberrations in human lymphocytes could not be reproduced by Mañas et al. (2009,

ASB2012-11892) who tested 96% analytical grade glyphosate up to a higher concentration of 6 mM.

Positive *in vitro* results were also reported when glyphosate was tested by means of (alkaline) single cell gel electrophoresis, i.e., in the Comet assay. In a study with "technical grade" glyphosate and a maximum concentration of 6.5 mM, Monroy et al. (2005, ASB2012-11910) observed an effect on the DNA in human fibroblasts and fibrosarcoma cells. Mañas et al. (2009, ASB2012-11892) found DNA damage in Hep-2 cells of human epithelial origin at glyphosate concentrations between 3 and 7.5 mM with the highest one being already cytotoxic. Mladinic et al. (2009b, ASB2012-11906) reported a similar effect in human lymphocytes without S9 mix at the highest concentration of 580 µg/mL (approx. 3.43 mM). With metabolic activation, tail length and intensity were increased even at a low concentration of 3.5 µg/mL and above. However, these findings were always accompanied by a high rate of early apoptotic and necrotic cells pointing to cytotoxicity. Alvarez-Moya et al. (2014, ASB2014-6902) who tested 96% glyphosate in human lymphocytes observed an increase in tail length at all tested concentrations from 0.7 up to 700 µM but the differences between the concentrations were surprisingly small and there was no clear dose response relationship. Koller et al. (2012, ASB2014-7618) investigated the effects of technical grade (95%) 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 but there was no dose response relationship indicating that the outcome was equivocal.

An overview on these studies is given in Table 22.

Table 22: Summary of *in vitro* tests for mutagenicity, clastogenicity or DNA damage/repair with glyphosate acid in mammalian cells

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels*; test conditions; purity	Results
Li, 1983; TOX9552369; Monsanto (also published by Li and Long, 1988, TOX9500253)	Mammalian cell gene mutation	Chinese hamster ovary (CHO) cells; HGPRT assay	- S9: 2 – 22.5 mg/mL + S9: 5 – 22.5 (25 ??) mg/mL; Lot XHJ-64; 98.7%	Negative
Jensen, 1991; TOX9552372; Cheminova	Mammalian cell gene mutation	Mouse lymphoma cells (L5178Y $TK^{+/-}$)	- S9: 0.61 – 5.0 mg/mL, + S9: 0.52 – 4.2 mg/mL; 98.6%	Negative
Clay, 1996, TOX2000-1994; Syngenta	Mammalian cell gene mutation	Mouse lymphoma cells (L5178Y $TK^{+/-}$)	+/- S9: 296 – 1000 µg/mL; P24; 95.6%	Negative
Van de Waart, 1995; TOX9651525; Agrichem	Chromosomal aberration	Peripheral human lymphocytes (-S9: 24, 48 h exposure; +S9: 3 h, harvest after 24 or 48 h)	- S9: 33 – 333 µg/mL + S9: 237 – 562 µg/mL; 96%	Negative (supplementary study)
Kyomu, 1995; ASB2012-11475; Arysta	Chromosomal aberration	Chinese hamster lung (CHL) cells	- S9: 62.5 – 500 µg/mL, + S9: 255 – 1000 µg/mL; 95.68%	Negative
Wright, 1996; ASB2012-11476; Nufarm	Chromosomal aberration	CHL cells	+/- S9: 312.5 - 1250 µg/mL; 95.3%	Negative
Fox, 1998; TOX2000-1995; Syngenta	Chromosomal aberration	Human lymphocytes	- S9: 100 – 1250 µg/mL + S9: 100 – 1250 µg/mL; 95.6%	Negative

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels*; test conditions; purity	Results
Lioi et al., 1998, ASB2013-9836	Chromosomal aberration	Bovine lymphocytes	-S9: 17 - 170 µM (3 - 30 µg/mL) +S9: not tested ≥ 98%	Positive (-S9)
Mladinic et al., 2009a, ASB2012-11907	Micronucleus formation	Human lymphocytes	-S9/+S9: 0.5 - 580 µg/mL 98%	Negative (-S9) Positive (+S9)
Mañas et al., 2009, ASB2012-11892	Chromosomal aberration	Human lymphocytes	-S9: 0.2-6.0 mM (34 - 1015 µg/mL) +S9: not tested 96%	Negative
Koller et al., 2012, ASB2014-7618	Micronucleus formation	Buccal carcinoma TR146 cells	10-20 µg/mL 95%	Positive
Rossberger, 1994; TOX9400697; Feinchemie (ADAMA)	UDS assay	Primary rat (Sprague-Dawley) hepatocytes	0.20 – 111.69 mM; >98%	Negative
Bolognesi et al., 1997, Z59299	Sister-chromatid exchange	Human lymphocytes	-S9: 0.33 and 6 mg/mL +S9: not tested 99.9%	Positive
Monroy et al., 2005, ASB2012-11910	Comet assay	Human fibroblast GM 39 and Human fibrosarcoma HT1080 cells	-S9 (GM39): 4.0-6.5 nM, -S9 (HT1080): 4.5-6.5 nM +S9: not tested Purity: not given	Positive
Mañas et al., 2009, ASB2012-11892	Comet assay	Human liver Hep-2 cells	-S9: 3 - 7.5 mM (507.2 - 1268 µg/mL) +S9: not tested 96%	Positive
Mladinic et al., 2009b, ASB2012-11906	Comet assay	Human lymphocytes	-S9/+S9: 0.5-580 µg/mL 98%	Positive
Koller et al., 2012, ASB2014-7618	Comet assay	Buccal carcinoma TR146 cells	10-2000 µg/mL 95%	Positive
Alvarez-Moya et al., 2014, ASB2014-6902	Comet assay	Human lymphocytes	-S9: 0.0007-0.7 mM (0.118- 118 µg/mL) +S9: not tested 96%	Positive

* Sometimes, higher concentrations were included in testing but these were the dose levels up to which analysis was carried out or reported.

On balance, regarding the *in vitro* studies with glyphosate, standard bacterial assays and mammalian cell gene mutation tests gave consistently negative results. Also, 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. More important, no evidence of chromosome aberration was obtained in a large number of higher tier *in vivo* studies that are described in the next sub-section. *In vitro* indicator tests gave positive results for induction of SCE and DNA strand breaks (comet assay) but a negative result for induction of DNA repair (UDS).

4.8.1.2 *In vivo* data

Extensive testing of glyphosate for mutagenicity was performed in vivo by means of micronucleus assays or chromosome aberration studies that all examined the bone marrow of either mice or rats after oral or intraperitoneal application. All these studies are summarised in Table 23, separated for the application route and the test species.

General suitability of the bone marrow examinations is shown by the affinity of glyphosate to bone tissue as shown in the ADME studies (see attached RAR, Vol. 3, B.6.1), by the occasional observation of bone marrow toxicity in the tests themselves (e.g., by Suresh et al, 1994, TOX9400323) and by the occurrence of hypoplasia in bone marrow in a long-term study in rats although this latter finding was confined to a very high dose (Wood et al., 2009; ASB2012-11490). Thus, there is sufficient evidence that the target tissue in these studies was actually exposed to the test compound.

In a total of 7 out of the 8 valid studies in Table 23, glyphosate of different manufacturing sources proved clearly negative. The only exception was a micronucleus test performed by Suresh (1993, TOX9551100) which demonstrated a statistically significant increase in the incidence of micronuclei in females but not in males at the very high dose of 5000 mg/kg bw that was administered on two consecutive days. In contrast, a cytogenetic study conducted in the same laboratory and the same mouse strain under nearly identical conditions did not provide any evidence of chromosome aberrations even though test material of the same purity was applied at the same dose levels (Suresh, 1994, TOX9400323). In this second study of the same group, a certain degree of cytoxicity to bone marrow cells at the highest dose level became apparent since the mitotic index was reduced. Although not measured in the preceding micronucleus test, such an effect could be expected to have occurred in the previous experiment, too, and cytotoxicity might have contributed to micronucleus formation. Last but not least, the study author also concluded that, under the conditions of the experiment, glyphosate was not mutagenic in the micronucleus test in mice.

A small number of manufacturers studies had been rejected by the DS because they were considered “not acceptable” due to serious deficiencies. One of these studies had caused some discussion during the ongoing evaluation process of glyphosate in the EU, in particular during the public consultation in 2014, since a “positive” result has been claimed. For consistency, this study is briefly reported here. Zoriki Hosomi (2007, ASB2012-11480) administered 98% pure glyphosate from a Brazilian manufacturer to male Swiss mice (six per dose level). The animals were dosed twice with a 24-hour interval between by oral gavage. Sampling took place 24 hours after the second dose. The dose levels were 8, 15, and 30 mg/kg bw, based on toxicity observed in a range-finding test. On bone marrow slides, 3000 PCE per animal were scored for micronuclei. At the highest dose level, there was a statistically significant increase in micronucleus frequency (Chi-square test, $p = 0.02$). Against the large database that is available for glyphosate, this finding is surprising, as well as the high toxicity. In the range finding experiment, two animals that had been administered 2000 mg/kg bw died on day 3 after having shown ataxia and prostration before. The same observations were made in 3 animals which received an oral dose of 320 mg/kg bw. They all died on day 2. Even at a dose level of 50 mg/kg bw, one out of three treated animals died on day 1. The occurrence of deaths and clinical signs at relatively low dose levels was obviously in contradiction to the available acute toxicity tests with glyphosate in the mouse (Komura, 1995, ASB2012-11382; Suresh, 1991, TOX9551089; Dideriksen and Skydsgaard, 1991, TOX9552329; Tos, 1994, TOX9551624) revealing an LD50 higher than 2000 or even 5000 mg/kg bw. In line with that, much higher dose levels were employed in the other (negative) micronucleus assays or cytogenetic studies in mice with substance administration by the oral route (see Table 23). To conclude, this study by Zoriki Hosomi (2007) was seriously flawed by severe toxicity that was completely unexpected and cannot be explained if the whole toxicological profile of glyphosate is taken into consideration. Either serious methodical mistakes have been made when the study was conducted or the test material was not glyphosate even though it was claimed as

such. Both possibilities would turn the study completely unreliable and make it unsuitable for any regulatory use.

Some more studies were performed by intraperitoneal application.

A statistically significant increase in micronucleated PCEs was observed by Durward (2006, ASB2012-11478) after single i.p. injection of 600 mg/kg bw to CD-1 mice. However, this response was modest and within the historical range for vehicle control animals and, therefore, was not considered biologically significant.

Mañas et al. (2009, ASB2012-11892) reported a positive result in a micronucleus test in bone marrow erythrocytes of *Balb* C mice (5 per dose, sex not stated). There was a statistically significant increase ($p < 0.01$ in Dunnett's test) in micronucleated cells at 24 hours after the animals had received two i.p. doses of 200 mg/kg bw, administered 24 h apart, of 96% analytical grade glyphosate. Two i.p. doses of 100 mg/kg bw each were without an effect. The result of this study is, however, flawed by major deviations from internationally agreed test guidelines: a) the sex of the animals was not reported, b) only 1000 (instead of 2000) erythrocytes per animal were scored, and c) "erythrocytes" instead of immature or "polychromatic erythrocytes" (PCE) were scored for micronuclei. In an assay with the reported treatment and sampling times, scoring of all erythrocytes instead of polychromatic erythrocytes is not appropriate according to OECD test guideline 474.

Bolognesi et al. (1997, Z59299) found a weak increase in micronuclei in mouse bone marrow following two i.p. doses of 150 mg/kg bw on two consecutive days. The test material was 99.9% (analytical grade) glyphosate. However, since only 3 or 4 animals were used in the dosed groups and no data for individual animals were provided, it is not possible to assess whether an outlier would have disproportionately influenced the result. In contrast, Rank et al. (1992, Z82234) did not observe an increase in micronucleated PCEs after single i.p. administration of up to 200 mg/kg bw of the glyphosate isopropylammonium (IPA) salt to mice with sampling after 24 and 48 hours. Similarly, Chruscielska et al. (2000, ASB2013-9830) reported a negative micronucleus assay in which glyphosate from Polish production was applied via the i.p. route at a single dose of 300 mg/kg bw to mice. All these studies had methodological deficiencies. The dose levels were lower than those used in the manufacturer's studies which were negative.

Furthermore, the oral route in the micronucleus assay or cytogenetic study is of higher relevance for risk assessment.

An overview of the valid micronucleus tests and cytogenetic studies *in vivo* is given in Table 23.

Table 23: Summary of somatic cell mutagenicity tests in mammals, *in vivo*

Reference	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results	GLP, Test guideline	Result details	Comments
Jensen, 1991, TOX9552374	Mouse, Micronucleus test, bone marrow	Glyphosate, 98.6% oral, 1x 0 or 5000 mg/kg bw, sampled after 24, 48 and 72 h	Negative	GLP, OECD 474 (1983)	*MN/2000 PCE [mean (range)]:* Control: 2.7 (1-4) 24h, 5000 mg/kg: 3.2 (1-5) 48h, 5000 mg/kg: 2.8 (1-6) 72h, 5000 mg/kg: 1.7 (0-4) PosControl: 48.2 (32-58)	5 animals per sex and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect.
Suresh, 1993, TOX9551100	Mouse, Micronucleus test, bone marrow	Glyphosate, 96.8% oral, 2x 0, 50, 500 or 5000 mg/kg bw (24 h interval), sampled 24 h after second dose	Weakly positive for top dose females	GLP, OECD 474 (1984)	*% MNPCE [mean (range)], male/female:* Control: 0.69 (0.1-1.6)/0.51 (0.2-1.0) 50 mg/kg: 0.84 (0.2-1.4)/0.28 (0.0-0.5) 500 mg/kg: 0.73 (0.4-1.6)/0.52 (0.2-1.3) 5000 mg/kg: 0.89 (0.7-1.1)/1.05*(0.4-1.6) PosControl: 2.33* (1.5-3.2)/2.39* (1.4-3.4) *p<0.05	5 animals per sex and dose (Control: 10/sex). 2000 PCE scored/animal. PCE/NCE: no effect (but PosControl).
Suresh, 1994, TOX9400323	Mouse, Chromosome aberration test, bone marrow	Glyphosate, 96.8% oral, 2 x 0-5000 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 475 (1984)	*No. of aberrations per 250-250-500 metaphases (male/female/total)* Control: 12/10/22 5000 mg/kg: 10/11/21 PosControl: 139*/155*/294* *p<0.05	5 animals per sex. 50 metaphases/animal examined. *Mitotic index (%) (male/female/total)* Control: 13.3/17.4/15.3 5000 mg/kg: 8.9*/9.5*/9.2* PosControl: 14.7/5.5*/10.1*
Fox & Mackay, 1996, TOX2000-1996	Mouse, Micronucleus test, bone marrow	Glyphosate, 95.6% oral, 1x 0 or 5000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/1000 PCE (mean±SD), male/female:* 24h, Control: 1.6±0.8/1.4±0.7 24h, 5000 mg/kg: 2.1±1.6/2.1±2.5 24h, PosControl: 22.2±6.1*/23.3±4.9* 48h, Control: 1.7 ±1.3/0.7±0.6 48h, 5000 mg/kg: 2.1±1.9/0.8±0.8 *p<0.01	5 animals per sex and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect.
Honarvar, 2008, ASB2012-11483	Mouse, Micronucleus test, bone marrow	Glyphosate, 99.1% oral, 1x 0, 500, 1000 or 2000 mg/kg bw, sampled after 24 h 1x 0 or 2000 mg/kg bw, sampled after 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)]:* 24h, Control: 1.4 (0-3) 24h, 500 mg/kg: 1.6 (1-2) 24h, 1000 mg/kg: 1.6 (1-2) 24h, 2000 mg/kg: 1.4 (0-2) 24h, PosControl: 63.0 (44-92)* 48h, Control: 1.4 (0-3)	5 males per group and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (293 studies): *% MNPCE [mean±SD, (range)]:*

Reference	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results	GLP, Test guideline	Result details	Comments
					48h, 2000 mg/kg: 1.6 (0-3) *p<0.01	*0.084±0.031 (0.01 – 0.18)*
Patel, 2012, ASB2014-9277	Mouse, Micronucleus test, bone marrow	Glyphosate, 98.9% oral, 2 x 0 or 2000 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*% MNPCE [mean (range)]:* Control: 0.033 (0-0.05) 2000 mg/kg: 0.0 (0-0) PosControl: 2.49* (1.1-3.7) *p<0.01	6 males per group. 2000 PCE scored/animal. PCE/NCE: no effect at 2000 mg/kg, increased in PosControl. Historical control data (of 73 studies) *% MNPCE [mean±SD (range)]:* *0.02±0.02 (0.0-0.07)*
Roth, 2012, ASB2014-9333	Mouse, Micronucleus test, bone marrow	Glyphosate, 96.3% oral, 1 x 0 or 2000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean±SD, (range)]:* 24h, Control: 3.2±3.6 (0-8) 24h, 2000 mg/kg: 2.3±0.5 (2-3) 24h, PosControl: 40.2±18.2* (16-67) 48h, Control: 1.4±1.1 (0-3) 48h, 2000 mg/kg: 1.1±1.3 (0-3) *p<0.01	7 males per group (Control and PosControl: 5 males each). 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (of 219 studies) *% MNPCE [mean±SD (range of mean group value)]:* 0.108±0.039 (0.01-0.25)
Flügge, 2009, ASB2012-11479	Rat, Micronucleus test, bone marrow	Glyphosate, 98.8% oral, 1 x 0, 500, 1000 or 2000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE (mean±SD), male/female:* 24h, Control: 1.6±1.1/1.8±0.4 24h, 500 mg/kg: 1.0±1.2/1.2±1.3 24h, 1000 mg/kg: 0.8±0.4/1.6±0.9 24h, 2000 mg/kg: 1.2±0.8/0.8±0.8 24h, PosControl: 30.2±10.5*/24.0±4.9* 48h, Control: 2.0 ±1.9/2.2 ±1.3 48h, 2000 mg/kg: 1.6±0.9/0.8±0.8 *p<0.05	5 animals per sex and dose and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (24, 48 and 72 h samplings combined): *MN/1000 PCE [mean and (range):* Males: 1.97 (0.4 – 5.7) Females: 1.86 (0.4 – 4.7)
Li and Long, 1988, TOX9500253 Li, 1983, TOX9552369	Rat, Chromosome aberration test, bone marrow	Glyphosate, 98% i.p., 1 x 0 or 1000 mg/kg bw, sampled after 6, 12 and 24 h	Negative	No GLP, no reference to TG	*% aberrant cells (mean), male/female/total:* 6h, Control: 1.3/2.7/2.0 6h, 1000 mg/kg: 2.3/3.0/2.7 12h, Control: 1.0/1.5/1.2 12h, 1000 mg/kg: 2.0/2.5/2.3 24h, Control: 1.3/2.3/1.8 24h, 1000 mg/kg: 1.0/3.7/2.6	Consistent with OECD 475 (1984): 6 animals per sex and sampling time. Ca 50 metaphases/animal examined. Slides were coded and scored "blind". Original study reported in RAR as

Reference	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results	GLP, Test guideline	Result details	Comments
					PosControl: 42.2*/23.8*/40.8* * p < 0.05	Li, 1983 (TOX9552375).
Rank et al., 1993, Z82234	Mouse, Micronucleus test, bone marrow	Glyphosate isopropylamine salt, purity not stated i.p., 1 x 0, 100, 150 or 200 mg/kg bw sampled after 24 and 48 h	Negative	No GLP, no reference to TG	*% MNPCE (mean±SD):* 24h, Control: 0.27±0.11 24h, 100 mg/kg: 0.20±0.13 24h, 150 mg/kg: 0.2±0.13 24h, 200 mg/kg: 0.25±0.10 24h, PosControl: 2.53±0.59 48h, 150 mg/kg: 0.13±0.09 48h, 200 mg/kg: 0.12±0.09	Consistent with OECD 474 (1983): Mostly 5 animals per sex and dose and sampling time. 1000 PCE scored/animal. Slides were scored randomly. PCE/NCE: no effect.
Bolognesi et al., 1997, Z59299	Mouse, Micronucleus test, bone marrow	Glyphosate, 99.9% i.p., 2 x 150 mg/kg bw (24 h interval), sampled 6 or 24 h after second dose	Positive	No GLP, no reference to TG	*MN/1000 PCE (mean±SD):* Control: 0.75±0.46 6h, 2x 150 mg/kg: 1.4±0.9 24h, 2x 150 mg/kg: 2.4±1.5* 24h, PosControl: 80.0±8.5* * p < 0.05	6 males in Control and PosControl group. 3000 PCE scored/animal. PCE/NCE: 0.73±0.06 in Control, 0.6±0.05 at 6h, 0.5±0.2 at 24h. *Deviations from OECD 474 (1997):* *Only 3(4) males examined per sampling time.* *Sampling time of Control not stated.* *Independent coding of slides not stated.*
Mañas et al., 2009a, ASB2012-11892	Mouse, Micronucleus test, bone marrow	Glyphosate, 96% i.p., 2 x 50, 100 or 200 mg/kg bw (24 h interval), sampled 24 h after second dose	Positive	No GLP, OECD 474 (1997)	*MN/1000* ***Erythrocytes*** *(mean±SD):* Control: 3.8 ±0.8 2x 50 mg/kg: 3.7±0.5 2x 100 mg/kg: 4.2±0.5 2x 200 mg/kg: 13.0±3.5* PosControl: 19.2±3.9* * *P* < 0.01	5 animals per dose. PCE/NCE no effect. *Deviations from OECD 474 (1997):* *Sex of animals not reported.* *1000* ***erythrocytes*** *(not* ***PCE****) scored/animal.* *Independent coding of slides not stated.*
Carvalho and Marques, 1999, ASB2012-11482	Mouse, Micronucleus test, bone marrow	Glyphosate, 95% i.p., 2 x 0, 187.5, 375 or 562.5 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, internal SOP	*MN/1000 PCE [mean (range)], male/female:* Control: 0.4 (0-1)/0.8 (0-2) 188 mg/kg: 0.0 (0)/0.6 (0-3) 375 mg/kg: 0.6 (0-3)/0.6 (0-2) 563 mg/kg: 0.4 (0-2)/0.6 (0-1) PosControl: 4.8* (4-7)/4.8* (2-12)	5 animals per sex and dose. 1000 PCE and 1000 NCE scored per animal. PCE/NCE: no effect (but PosControl). MN/1000 NCE: no effect (but

Reference	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results	GLP, Test guideline	Result details	Comments
					*p<0.05	PosControl). *$LD50_{i.p.}$=750 mg/kg*
Durward, 2006, ASB2012-11478	Mouse, Micronucleus test, bone marrow	Glyphosate, 95.7% i.p., 1 x 0, 150, 300 or 600 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*% MNPCE [mean±SD, (range)]:* 24h, Control: 0.06±0.06 (0.0-0.15) 24h, 150 mg/kg: 0.07±0.04 (0.0-0.10) 24h, 300 mg/kg: 0.06±0.05 (0.0-0.15) 24h, 600 mg/kg: 0.19±0.07* (0.05-0.25) 24h, PosControl: 3.03±0.49*** (2.20-3.35) 48h, Control: 0.1±0.12 (0.0-0.35) 48h, 600 mg/kg: 0.09±0.11 (0.0-0.30) *p<0.05, ***p<0.001	7 males per group and sampling time. 2000 PCE scored/animal. *Pre-test: Mortality at 800-1000 mg/kg, clinical signs at 150 mg/kg and above.* PCE/NCE: reduced at 600 mg/kg (not in PosControl). Stat. sign. increase in MNPCE at 600 mg/kg (24 h), within historical control. *Control data from 60 groups (24h):* *0.0-0.9 MN/1000 PCE: 40x (67%)* *1.0-1.4 MN/1000 PCE: 14x (23%)* *1.5-2.0 MN/1000 PCE: 3x (5%)* *2.1-2.5 MN/1000 PCE: 3x (5%)*
Costa, 2008, ASB2012-11481	Mouse, Micronucleus test, bone marrow	Glyphosate, 98% i.p., 2 x 0, 15.6, 31.3 or 62.5 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)], male/female:* Control: 0.0 (0)/0.0 (0) 15.6 mg/kg: 0.0 (0)/0.0 (0) 31.3 mg/kg: 0.0 (0-1)/0.0 (0) 62.5 mg/kg: 0.6 (0-3)/0.0 (0) PosControl: 23.0* (8-30)/12.2* (7-26) *p<0.01	5 animals per sex and dose. 2000 PCE scored/animal. *Pre-test: Mortality at 500-1000 mg/kg, decreased PCE/NCE at 250 mg/kg and above.* PCE/NCE no effect. Historical control: ca. 3 MN/1000 PCE
Costa, 2010, ASB2014-9284	Mouse, Micronucleus test, bone marrow	Glyphosate, 98% i.p., 2 x 0, 125, 250 or 375 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)], male/female:* Control: 0.4 (0-2)/0.4 (0-1) 125 mg/kg: 0.2 (0-1)/0.0 (0-1) 250 mg/kg: 0.0 (0)/0.0 (0) 375 mg/kg: 0.2 (0-1)/0.0 (0-1) PosControl: 8.0* (5-11)/6.4* (5-9) *p<0.01	5 animals per sex and dose. 2000 PCE scored/animal. *Clinical signs at 125 mg/kg and above.* PCE/NCE: slight increase at 250 and 375 mg/kg and in PosControl. Historical control: ca. 3 MN/1000 PCE

NCE, normochromatic erythrocytes; MN, micronucleus; MNPCE%, percent of micronucleated polychromatic erythrocytes; PCE, polychromatic erythrocytes; SD, standard deviation

Table 24: Summary of tests on DNA adducts and DNA strand breaks in mammals, *in vivo*

Reference	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments
Bolognesi et al., 1997, Z59299	Mouse DNA adduct (8-OHdG by LC/UV), liver	Analytical grade glyphosate (purity 99.9%) i.p.; 1 × 300 mg/kg bw; sampled after 8 and 24 h	- (4 h) + (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 0.6 moles 8-OHdG/10^5 moles dG 4 h: approx. 0.9 moles 8-OHdG/10^5 moles dG 24 h: approx. 3.6 moles 8-OHdG/10^5 moles dG*	3 male animals per group, at least 3 independent repeat experiments
Bolognesi et al., 1997, Z59299	Mouse DNA adduct (8-OHdG by LC/UV), kidney	Analytical grade glyphosate (purity 99.9%) i.p.; 1 × 300 mg/kg bw; sampled after 8 and 24 h	- (4 & 24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 0.6 moles 8-OHdG/10^5 moles dG 4 h: approx. 0.5 moles 8-OHdG/10^5 moles dG 24 h: approx. 0.4 moles 8-OHdG/10^5 moles dG*	3 male animals per group, at least 3 independent repeat experiments
Peluso et al., 1998, TOX1999-318	Mouse DNA adduct (^{32}P-DNA post labelling), kidney	Glyphosate isopropylammonium salt i.p.; 1 × 0, 130 or 270 mg/kg bw; sampled after 24 h	–	No GLP, no reference to TG	Not reported	6 animals in control group, 6 in low dose group and 3 in high dose group, sex of animals not clear
Peluso et al., 1998, TOX1999-318	Mouse DNA adduct (^{32}P-DNA post labelling), liver	Glyphosate isopropylammonium salt i.p.; 1 × 0, 130 or 270 mg/kg bw; sampled after 24 h	–	No GLP, no reference to TG	Not reported	6 animals in control group, 6 in low dose group and 3 in high dose group, sex of animals not clear
Bolognesi et al., 1997, Z59299	Mouse DNA strand breaks (alkaline elution assay), liver	Analytical grade glyphosate (purity 99.9%) i.p.; 1 × 300 mg/kg bw; sampled after 4 and 24 h	+ (4 h) - (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 15 *10^3/mL 4 h: approx. 47 *10^3/mL* 24 h: approx. 20 *10^3/mL	3 male animals per group, at least 4 independent repeat experiments
Bolognesi et al., 1997, Z59299	Mouse DNA strand	Analytical grade glyphosate (purity 99.9%)	+ (4 h) - (24 h)	No GLP, no	(Estimated from figure in report)	3 male animals per group, at least 4

Reference	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments
	breaks (alkaline elution assay), kidney	i.p.; 1 × 300 mg/kg bw; sampled after 4 and 24 h		reference to TG	Control: approx. 17 *10^3/mL 4 h: approx. 55 *10^3/mL* 24 h: approx. 25 *10^3/mL	independent repeat experiments
Manas et al., 2013, ASB2014-6909	Mouse comet assay, blood cells	Glyphosate (96%) Drinking water, 14 days, 0, 40 or 400 mg/kg bw per day; sampled after treatment period	+	No GLP, no reference to TG	Tail moment (mean ± SEM): Control: 2.98±1.08 40 mg/kg bw per day: 8.54***±7.82 400 mg/kg bw per day: 9.06***±5.15	6 animals per group sex of animals not clear
Manas et al., 2013, ASB2014-6909	Mouse comet assay, liver cells	Glyphosate (96%) Drinking water, 14 days, 0, 40 or 400 mg/kg bw per day; sampled after treatment period	+	No GLP, no reference to TG	Tail moment (mean ± SEM): Control: 7.14±3.41 40 mg/kg bw per day: 7.92*±3.99 400 mg/kg bw per day: 20.59***±15.47	6 animals per group sex of animals not clear

8-OHdG, 8-hydroxy-2' -deoxyguanosine; dG, deoxyguanosine; SEM, standard error of the mean; SCGE, single cell gel electrophoresis

Apart from this study type, there is some rather equivocal published information that was gained by other methods.

A possible impact on the DNA was investigated by Bolognesi et al. (1997, Z59299) also in vivo. A transient but significant effect towards DNA damage in liver and kidney was noted in the alkaline elution assay after glyphosate (300 mg/kg bw) had been administered once by the i.p. route to mice. This assay may indicate the induction of DNA single-strand breaks and alkali labile sites. A test for DNA oxidative damage suggested glyphosate to stimulate oxidative metabolism in the liver at 24 hours after application. This data is not easy to interpret since the results are given in summary figures only which are based on pooled individual data. There are reporting inconsistencies, e.g., it is not clear how many animals were actually used for testing. A positive control substance was not included. In contrast, no evidence for DNA adduct formation was reported following intraperitoneal administration of glyphosate isopropylammonium salt to mice at a single dose of 270 mg/kg bw (Peluso et al., 1998, TOX1999-318).

More recently, Mañas et al. (2013, ASB2014-6909) reported a positive Comet assay in liver and blood cells of *Balb C* mice after glyphosate (96% analytical grade) administration at dose levels of 40 and 400 mg/kg bw/day for 14 days in drinking water. A clear dose response was seen only in the liver. The authors also reported evidence of oxidative stress.

Taking into account that glyphosate proved negative in the UDS assay (Rossberger, 1994, TOX9400697), the published findings in this indicator test are not considered to provide convincing evidence of an interaction with the DNA. Positive results in the alkaline elution assay may also occur as a result of toxic but non-mutagenic effects. In general, DNA damage end points such as SCE or alkaline SCGE are generally regarded as supplementary to the gene mutation and chromosome effects end point categories. DNA damage endpoints do not directly measure effects on heritable mutations or events closely associated with chromosome mutations. Stimulation of oxidative metabolism is not a sign of mutagenicity but may elucidate a possible mechanism behind toxic effects.

4.8.2 Human information

There is (partly contradictory) epidemiological data available that should be used, however, with some reservation. It must be taken into account that the study participants had been always exposed to plant protection products containing glyphosate but never to the active substance itself. Furthermore, there must have been parallel exposure to many other environmental chemicals. Thus, the situation resembles that one for many chemicals. In the "Guidance on the Application of the COP Criteria (Version 4.1, June 2015), it is stated therefore: "Epidemiological studies have been to date unable to provide evidence to classify a substance as a Category 1A mutagen."

For the available data, the reader is referred to Vol. 3 of the attached RAR, Section B.6.4.8.7.

4.8.3 Other relevant information

Not available.

4.8.4 Summary and discussion of mutagenicity

Glyphosate has been tested in an adequate range of mutagenicity and genotoxicity tests.

In vitro bacterial assays and mammalian cell gene mutation assays gave consistently negative results. Also, results from *in vitro* mammalian chromosome aberration tests and *in vitro* micronucleus tests were negative when the studies were conducted according to internationally agreed test guidelines. *In vitro* indicator tests for induction of SCE and DNA strand breaks gave positive results.

In vivo, 11 micronucleus tests or cytogenetic studies in somatic cells that were conducted according to internationally agreed test guidelines gave negative results, while in only one test a weakly positive effect was seen in female mice receiving a very high and likely cytotoxic dose. Published studies with methodological limitations revealed contradictory results. In most of these studies, relatively low dose levels were employed and the intraperitoneal route was used which does not properly reflect the human exposure. When the weight of evidence is considered, it can be concluded that glyphosate was devoid of a clastogenic potential. Evidence of DNA damage such as strand breaks was observed in several published indicator tests following a high i.p. dose or repeated oral (via drinking water) doses. In contrast, an UDS was negative. Usually, standard mutagenicity tests such as cytogenicity or micronucleus assays are considered more important than indicator tests.

As reported in the beginning of this section, there was no evidence for mutagenic activity in germ cells of mice and rats at oral doses up to 2000 mg/kg bw.

In summary, taking a weight of evidence approach, glyphosate (active substance) is considered not mutagenic.

4.8.5 Comparison with criteria

The following criteria for classification for germ cell mutagens are given in the CLP regulation:

CLP regulation
The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1B is based on: — positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or — other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

There is no positive evidence of mutagenicity/genotoxicity coming from epidemiological studies.

Accordingly, category 1A is clearly not appropriate. Likewise, because of the negative results in the majority of the *in vitro* and *in vivo* mutagenicity tests including nearly all guideline-compliant standard assays and since positive findings were mainly confined to indicator tests, categories 1B and 2 also do not apply.

4.8.6 Conclusions on classification and labelling

No hazard classification of glyphosate for mutagenicity is warranted according to the CLP criteria.

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 ^{32}P-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 gentotoxicity 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 challeging 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.

<u>Supporting evidence/indicator tests:</u>

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.

4.9 Carcinogenicity

4.9.1 Non-human information

Long-term toxicity and carcinogenicity of glyphosate were investigated in a large number of studies in rats and mice that are all tabulated in this section, first those in rats and subsequently those in mice. Published data is reported below the tables. Thereafter, tumour types of which the incidence was increased in at least one study in the respective species are considered in detail.

Studies in rats

The DS is aware of a total of 9 unpublished long-term feeding studies with the technical active ingredient in rats (Table 25) of which 6 were performed in compliance with OECD TG 453 whereas the remaining three were flawed by serious deficiencies. The main effects as summarised in this table were statistically significant and either dose-related or observed at the top dose level only. However, they were not necessarily all noted at the LOAEL. Two more (published) studies with a glyphosate salt and a formulation are briefly reported below the table.

Table 25: Long-term feeding studies with glyphosate in rats (deficient studies on bottom)

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration	Dose levels	NOAEL	LOAEL	Targets / Main effects
Wood et al., 2009; ASB2012-11490; H05H016A, 95,7%; Nufarm	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Wistar	0, 1500, 5000, 15000 ppm (progressively increased up to 24000 ppm), equal to 86/105, 285/349, and 1077/1382 mg/kg bw/d (m/f)	285 mg/kg bw/d	1077 mg/kg bw/d	Bw gain↓, transient increase in AP activity, changes in distribution of renal mineralisation, adipose infiltration of bone marrow (indicative of hypoplasia)↑, slight increase in cutaneous alterations
Brammer, 2001; ASB2012-11488; P30, 97.6%; Syngenta	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Wistar-derived	0, 2000, 6000, 20000 ppm (121/145, 361/437, 1214/1498 mg/kg bw/d in m/f)	361 mg/kg bw/d	1214 mg/kg bw/d	Bw, food consumption and (initially) utilization↓, clinical chemistry findings (AP and ALAT activity↑, bilirubin↑, urine pH↓), kidney papillary necrosis, prostatis and periodontal inflammation↑ in high-dose males
Enomoto, 1997; ASB2012-11484, 11485, 11486, 11487; T-941209, 97.56% and T-950308, 94.61%; Arysta	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Sprague-Dawley	0, 3000, 10000, 30000 ppm (104/115, 354/393, 1127/1247 mg/kg bw/d in m/f)	104 mg/kg bw/d	354 mg/kg bw/d	Bw/bw gain, food consumption (initially) and utilization↓, loose stool↑, tail masses↑ due to follicular hyperkeratosis and abscesses, caecum: distention and wt↑, pH↓ and dark appearance of urine
Suresh, 1996; TOX9651587; 2 batches used,	Combined chronic toxicity/	0, 100, 1000, 10000 ppm (6.3/8.6, 59.4/88.5,	59 mg/kg bw/d	595 mg/kg bw/d	AP activity↑ (f), slight in-crease in cataracts (m, no clear dose response in f)

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration	Dose levels	NOAEL	LOAEL	Targets / Main effects
96.8/96.0%; ADAMA	carcino-genicity (OECD TG 453); 2 yr; Wistar	595.2/886 mg/kg bw/d in m/f)			
Atkinson et al., 1993; TOX9750499; 229-JaK-5-1, 98.9% and 229-JaK-142-6, 98.7%; Cheminova	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Sprague-Dawley	0, 10, 100, 300, 1000 mg/kg bw/d (dietary levels regularly adjusted)	100 mg/kg bw/d	300 mg/kg bw/d	Bw gain↓, AP activity↑, urine pH↓, salivary glands: wt↑ and histological findings, liver wt↑
Stout and Ruecker, 1990; TOX9300244; XLH-264, 96.5%; Monsanto	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Sprague-Dawley	0, 2000, 8000, 20000 ppm (89/113, 362/457, 940/1183 mg/kg bw/d in m/f)	89 mg/kg bw/d	362 mg/kg bw/d	Bw and bw gain↓ in f, liver wt↑, stomach mucosal inflammation, cataracts in m, urine pH↓, survival <50% in all groups incl. controls
Bhide, 1997*; ASB2012-11489	Combined chronic toxicity/ carcinoge-nicity; 2 yr; Sprague-Dawley	0, 3000, 15999, 25000 ppm (150/210, 780/1060, 1290/1740 mg/kg bw/d in m/f)	150 mg/kg bw/d	780 mg/kg bw/d	AP activity↑ (m/f), bw gain↓ in m, equivocal alterations in organ weights (testis, brain, liver, kidneys) mostly at interim sacrifice (after 1 yr)
Lankas, 1981**; TOX2000-595 and TOX2000-1997; XHJ-64, 98.7%; Monsanto	Combined chronic toxicity/ carcinoge-nicity; 26 months; Sprague-Dawley	0, 3/3.4, 10.3/11.2, 31.5/34 mg/kg bw/d in m/f (dietary levels adjusted according to values as measured in the 1st week)	31.5 mg/kg bw/d (NOEL)	Not estab-lished	No effects observed
Calandra, 1974***; Z35230; Monsanto	Chronic toxicity study; 2 yr; "Charles River albino rat"	0, 30, 100, 300 ppm	100 ppm	300 ppm	Liver (lipidosis)

*poor study with many serious reporting deficiencies including lacking information on test material, surprisingly low spontaneous tumour incidences in the controls but the number of animals undergoing histopathology was also low; study rejected for EU risk assessment process; **study flawed by serious reporting deficiencies and employment of too low dose levels far below an MTD, not acceptable according to current standards but previously often used for regulatory purposes; ***deficient IBT study, not guideline-compliant, dose levels much too low for meaningful evaluation, not used for any regulatory assessment during the last decades

In a published study (Chruścielska et al., 2000a; ASB2013-9829), administration of glyphosate was also oral but via drinking water. A 13.85% aqueous solution of glyphosate ammonium salt (purity and batch not given in the article) was administered for two years to Wistar-RIZ outbred rats at

concentrations of 300, 900, or 2700 mg/L. The initial group size was very large with 85 male and female rats per dose level of which 30 animals in total (i.e., 10 per timepoint) per dose and sex were used for interim sacrifices after 6, 12, or 18 months of treatment. It was stated that the study was conducted in compliance with OECD 453 but the report is very brief and no raw data is available. There was no increase in neoplastic lesions neither in males nor in females at any dose level as demonstrated in two tables displaying the cancer incidences. Due to reporting deficiencies and because a glyphosate salt solution but not the acid was tested, this study if of very limited value with regard to classification and labelling.

A further two-year study in rats was published by Séralini et al. (2012, ASB2012-15514) but a formulation and not the active substance was tested. Its main objective was to investigate a possible impact of long-term feeding of genetically modified (glyphosate-resistant) maize to rats but three of the test groups were administered a commercially available formulation (Roundup GT Plus, apparently authorised at least in Belgium) containing 450 g glyphosate/L at different concentrations ranging from 0.1 ppb (50 ng glyphosate/L) to 0.5% (2.25 g glyphosate/L) in drinking water. In these groups, the authors reported alterations in some clinical chemistry (blood and urine) parameters and hormone levels and histopathological lesions concerning the liver and the gastrointestinal tract but also a higher incidence of mammary tumours in females resulting in a shorter lifespan. This study was heavily discussed in the scientific community as well as in the general public where it gained notable attention due to massive promotion although it was clearly flawed by many serious deficiencies. A major point of concern was the small group size of only 10 males and 10 females per dose, i.e., the test design was that of a subchronic study. Such a small number of animals is not sufficient for a long-term study because age-related changes cannot be adequately taken into account. A comprehensive critical assessment of this study was published by EFSA (2012, ASB2012-15513). The conclusion was that: "the currently available evidence does not impact on the ongoing re-evaluation of glyphosate […]". Later on, the paper was withdrawn by the journal in which it had been first published but was re-published in another one. In any case, this study is not suitable for classification and labelling purposes.

Because of the strong limitations of the two published studies, evaluation of carcinogenicity of glyphosate to rats can be based only on the studies that are summarised in Table 25. Due to their deficiencies, also the studies by Bhide (1997, ASB2012-11489), by Calandra (1974, Z35230) and by Lankas (1981, TOX2000-595 and TOX2000-1997) cannot be considered suitable for this purpose. However, since the latter study was subject to debate with regard to certain tumour types, it is taken here into consideration, along with the 6 guideline-compliant studies.

According to the evaluation by the DS, no evidence of carcinogenicity was obtained in any of the long-term studies in rats. Chronic toxicity was confined to high dose levels in all the studies but clear differences became apparent in what was actually observed (see Table 25). For more information, the reader is referred to the attached RAR (Volume 1, 2.6.6.1; Volume 3, B.6.5.1).

However, in the public debate on glyphosate but also in the IARC evaluation (IARC, 2015, ASB2015-8421), some neoplastic findings in two older studies have been subject to discussion. These findings comprised:

- an increase in islet cell tumours of the pancreas in both of these studies (Stout and Ruecker, 1990, TOX9300244; Lankas, 1981, TOX2000-595, TOX2000-1997)
- an increase in liver tumours in the study by Stout and Ruecker (1990, TOX9300244);
- an increase in C-cell adenoma of the thyroid in the same study; and
- an increase in interstitial cell tumours of the testis in the study by Lankas (1981, TOX2000-595, TOX2000-1997).

In the following, all these tumour types are considered in greater detail. That means also that the statistical calculations were repeated. In the original study reports, mostly pairwise comparisons had been made. In the 2015 IARC evaluation, trend tests were the preferred statistical tool. The DS re-calculated the statistical significance of the observed tumour incidences by taking both approaches.

For overall assessment, however, it must be further acknowledged that glyphosate is different from most other active substances in plant protection products because a number of comprehensive and high quality studies are available for nearly all toxicological endpoints. If dose levels are comparable, it would be expected that adverse effects were, at least to a certain extent, reproducible in other studies. A "weight of evidence" approach should and may be applied, therefore, as a general principle. Findings (including neoplastic) will be considered to have occurred by chance if they are not dose-related or cannot be confirmed at higher dose levels in other studies.

Pancreatic islet cell tumours

IARC noted that, based to the tumour incidences reported by Stout and Ruecker (1990, TOX9300244), a significant increase in pancreatic islet cell adenoma in male rats was observed at two dose levels but there were neither a statistically significant positive trend nor a progression to carcinoma. When the DS re-evaluated the reported incidences using Cochran-Armitage trend testing, the absence of a statistically positive trend was confirmed (Table 26).

The pairwise comparison by Fisher's exact test, in contrast, revealed a significant increase over the control incidence but only for the low dose group. Apparently, there was no clear dose response, which one would expect. Indeed, there was no progression towards malignancy since the only carcinoma in this study was found in a control male.

Table 26: Pancreatic islet cell tumours in SD rats (Stout and Ruecker, 1990, TOX9300244). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values for the pairwise comparison reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males/Group	Animals with islet cell adenoma
0	43	1
89	45	8 (0.030)
362	49	5 (0.209)
940	48	7 (0.062)
Trend test (p-value)		0.1687

In addition, IARC reported a significant increase in the incidence of pancreatic tumours in a second study in SD rats, i.e., in one of the treated male groups in the study of Lankas (1981, TOX2000-595, TOX2000-1997). However, according to IARC, there was no positive trend over all dose groups and, again, no indication for progression to carcinoma. Re-evaluation by the DS confirmed a significant increase in adenomas and for adenomas and carcinomas combined for the male low dose group when compared to the concurrent controls. Pairwise comparison did not reveal statistical significance for the pancreatic islet cell adenoma at the two upper dose levels. However, a significantly positive trend for carcinomas in male animals was found that has not been previously reported (Table 27). There was no increase in pancreatic tumours in the females.

Table 27: Pancreatic tumours in male SD rats (Lankas, 1981, TOX2000-595, TOX2000-1997). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males/Group	Adenoma	Carcinoma	Adenoma + Carcinoma
0	50	0	0	0
3	49	5 (0.027)	0 (1.000)	5 (0.027)
10.3	50	2 (0.495)	0 (1.000)	2 (0.495)
31.5	50	2 (0.495)	1 (1.000)	3 (0.242)
Trend test (p-value)		0.5284	0.0496	0.3207

This situation is similar as in the study by Stout and Ruecker (1990, TOX9300244). There was evidence of an increase in pancreatic tumours in treated males but, again, the difference to the control group was strongest in the low dose group and a clear dose response was missing. The positive trend for carcinoma in this study is due to the rare occurrence of this tumour and the incidence of a single carcinoma in the high dose group compared to the absence of this tumour type in the control and lower dose groups.

For overall assessment, it must be taken into consideration that in the five more recently conducted and guideline-compliant rat studies summarised in Table 25, even at very high dose levels, no increase in pancreas tumours was seen (Table 28). In four of them, incidence was highest in the control group. In the two studies discussed above, the incidences were elevated in treated groups but without a clear dose response.

Table 28: Pancreatic islet-cell tumours in long-term studies with glyphosate in male rats

Study	Control	Low dose	Mid dose	Second mid dose	High dose
Wood et al., 2009, ASB2012-11492	4 / 51	1 / 51 (86 mg/kg bw/day)	2 / 51 (285 mg/kg bw/day)	-	1 / 51 (1077 mg/kg bw/day)
Brammer et al., 2001, ASB2012-11488	1 / 53	2 / 53 (121 mg/kg bw/day)	0 / 53 (361 mg/kg bw/day)	-	1 / 52 (1214 mg/kg bw/day)
Enomoto, 1997, ASB2012-11484, 11485, 11486, 11487; T-941209	4 / 50	1 / 50 (104 mg/kg bw/day)	2* / 50 (354 mg/kg bw/day)	-	1 / 50 (1127 mg/kg bw/day)
Suresh, 1996, TOX9651587	3 / 48	0 / 30 (6.3 mg/kg bw/day)	0 / 32 (59.4 mg/kg bw/day)	-	1 / 49 (595.2 mg/kg bw/day)
Atkinson et al., 1993, TOX9552382	7 / 50	1 / 24 (10 mg/kg bw/day)	2 / 17 (100 mg/kg bw/day)	2 / 21 (300 mg/kg bw/day)	1 / 49 (1000 mg/kg bw/day)

Study	Control	Low dose	Mid dose	Second mid dose	High dose
Stout and Ruecker, 1990, TOX9300244	2* / 43	8 / 45 (89 mg/kg bw/day)	5 / 49 (362 mg/kg bw/day)		7 / 48 (940 mg/kg bw/day)
Lankas, 1981, TOX2000-595, TOX2000-1997	0 / 50	5 / 49 (3 mg/kg bw/day)	4 / 50 (10.3 mg/kg bw/day)	-	3* / 50 (31.5 mg/kg bw/day)

*including one carcinoma

To conclude, an (occasionally significant) increase in pancreatic tumours in male rats was confined to two studies of which one is now considered insufficient due to the very low doses employed and because of reporting deficiencies. In both cases, a dose-response was lacking and there was no tendency of progression to malignant neoplasia. A higher incidence of pancreatic tumours was not reproducible in five more recent, guideline-compliant studies with a spontaneous incidence in untreated control animals that sometimes resembled the frequencies that were reported by Stout and Ruecker (1990, TOX9300244) or Lankas (1981, TOX2000-595, TOX2000-1997).

Liver tumours

In the study of Stout and Ruecker (1990, TOX9300244), again, IARC reported a significantly positive trend for hepatocellular adenoma in males (Table 29). When the reported incidences were re-evaluated by the DS using Cochran-Armitage trend testing and Fisher's exact test, the statistically positive trend was confirmed for adenomas but no positive trend was observed for adenoma and carcinoma combined. In particular for combined incidence, a dose response was hardly to be seen and the pairwise comparison failed to reveal a statistically significant difference between any of the treated groups and the control group.

Table 29: Liver cell tumours in male SD rats (Stout and Ruecker, 1990, TOX9300244). Fisher's exact test was used to compare each treatment group to control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	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)
Trend test (p-value)		0.0171	0.0752

Moreover, no increase in liver tumours was reported in any other long-term study in rats. In general, hepatotoxicity of glyphosate is very limited. In fact, absolute and relative liver weight was increased in high dose males in the study by Stout and Ruecker (1990, TOX9300244) but there were no pre-neoplastic findings that might progress to liver tumours. Based on the lack of increased liver tumour rates in all other long-term/carcinogenicity studies in two rat strains (Wistar and SD), the DS interpreted the increased incidence of liver tumours, mainly due to increased rates of liver adenomas,

in one study as not attributable to glyphosate but to have occurred by chance.

Thyroid C-cell tumours

In the study of Stout and Ruecker (1990, TOX9300244), there was an increase in C-cell adenoma in female rats. This tumour was detected in 2 control and 2 low dose females but in 6 animals of the mid and high dose group each. In contrast to the (negative) pairwise comparison, the Cochran-Armitage trend test was weakly positive ($p = 0.0435$). In the absence of such a finding in any of the other rat studies, this increase in C-cell tumours is also considered a chance event. In addition, the thyroid is not a target organ of glyphosate. There were neither an increase in pre-neoplastic histological lesions nor an organ weight change noted in any other study with glyphosate even though distribution of radiolabelled glyphosate to the thyroid has been demonstrated in ADME studies by Ridley and Mirly (1988, TOX9552356) and by McEwen (1995, ASB2012-11379).

Interstitial cell tumours of the testes

In the study by Lankas (1981, TOX2000-595, TOX2000-1997), an increase of interstitial testicular tumours was observed. The actual incidences were 0/50, 3/50, 1/50, and 6/50 animals in the control group and at the three dose levels, respectively. Apparently, there was no clear dose response but in the top dose group receiving ca 31.5 mg glyphosate/kg bw per day, the difference to the control was statistically significant (Fisher's exact test, $p < 0.05$). In the original study report, it was argued that the absence of this tumour type in the control group was unusual and that the top dose incidence was only marginally above the historical control range. Reliability of this information could not be verified and, even if correct, this explanation would not be convincing. However, and more important, no increase in testicular tumours was observed in any other long-term study with glyphosate in rats even though much higher doses were administered.

Studies in mice

In total, five long-term studies are available that may be considered valid according to current standards and were performed in compliance with OECD TG 451. They are summarised in Table 30. As in rats, chronic toxicity was confined to high dose levels in all the studies but some differences became apparent in what was actually observed. For more information, the reader is referred to the attached RAR (Volume 1, 2.6.6.2, Volume 3, B.6.5.2).

The DS is aware of two further long-term studies in mice which have been very briefly reported in an older EU evaluation report (Germany, 1998, ASB2010-10302). These studies by Vereczkey and Csanyi (1982, TOX9650154) and by Bhide (1988, TOX9551831) did not comply with current standards. In both of them, the top dose level was 300 ppm and, thus, much too low for meaningful evaluation. No increase in any tumour type had been reported but these studies are not suitable for the purpose of classification and labelling. The same holds true for a published study on skin tumour promotion (George et al., 2010, ASB2012-11829). This experiment was performed with a commercial product that most likely contains irritating co-formulants. It cannot contribute to a decision on the classification of glyphosate. Furthermore, the up- and down-regulation of protein expression is not sufficient to prove a carcinogenic effect. Apart from that, there are no published studies on carcinogenicity in mice.

Thus, evaluation of a carcinogenic potential of glyphosate in mice is based on the five available, guideline-compliant studies. In line with the approach taken for the rat studies, the main effects as summarised in this table were statistically significant and either dose-related or observed at the top

dose level only. This approach implies that these findings were not necessarily all noted at the LOAEL.

Table 30: Long-term feeding studies with glyphosate in mice

Reference; Study identifi-cation; purity; Owner	Study type, strain, duration, route	Dietary dose levels and corresponding mean daily intake	NOAEL	LOAEL	Targets / Main effects
Wood et al., 2009, ASB2012-11492; 95.7%; Nufarm	Carcinogenicity (OECD TG 451); 18 mo; CD-1 (ICR), feeding	0, 500, 1500, 5000 ppm (71/98; 234/299; 810/1081 mg/kg bw/d in m/f)	810 mg/kg bw/d	Not established	No effects observed
Kumar, 2001, ASB2012-11491; >95.14%; ADAMA	Carcinogenicity (OECD TG 451); 18 mo, Swiss albino	0, 100, 1000, 10000 ppm (15; 151; 1460 mg/kg bw/d, sexes combined since values were similar)	151 mg/kg bw/d	1460 mg/kg bw/d	Higher incidence of malignant lymphoma at top dose level (outside historical control range for males); cystic glands in stomach in m↑ (equivocal toxicological relevance)
Sugimoto, 1997, ASB2012-11493; 97.56% or 94.61% (2 lots used); Arysta	Carcinogenicity (OECD TG 451); 18 mo; CD-1 (ICR)	0, 1600, 8000, 40000 ppm (165/153; 838/787; 4348/4116 mg/kg bw/d in m/f)	153 mg/kg bw/d	787 mg/kg bw/d	Bw gain, food consumption and efficiency↓, loose stool, caecum distended and organ wt↑, prolapse and ulceration of anus in m
Atkinson et al., 1993; TOX9552382; 98.6%; Cheminova	Carcinogenicity (OECD TG 451); 2 yr, CD-1	0, 100, 300, 1000 mg/kg bw/d (dietary levels regularly ad-justed)	1000 mg/kg bw/d	Not established	Equivocal evidence of enlarged/firm thymus and increase in mineral deposition in the brain, not regarded as adverse
Knezevich and Hogan, 1983; TOX9552381; 99.7%; Monsanto	Carcinogenicity with chronic to-xicity elements (OECD TG 451/453); 2 yr, CD-1	0, 1000, 5000, 30000 ppm 157/190; 814/955; 4841/5874 mg/kg bw/d in m/f)	157 mg/kg bw/d	814 mg/kg bw/d	Bw (gain) ↓ in high dose males, histological findings in liver (centrolobular hy-pertrophy), kidney (histological changes) and bladder (epithelial hyperplasia) in males

In these studies, there was evidence of increases in three types of tumours, all in males: malignant lymphoma, renal tumours, and haemangiosarcoma, however, there was no consistency between the studies. In the following, all these three types are addressed in detail. That means also that the statistical calculations were repeated. In the original study reports, mostly pairwise comparisons had been made. In the 2015 IARC evaluation, in contrast, trend tests were the preferred statistical tool. The DS re-calculated the statistical significance of the observed tumour incidences by taking both approaches.

Malignant lymphoma

The total numbers of affected animals in the various mouse studies are given in Table 31.

Table 31: Total incidence of malignant lymphoma in long-term studies with glyphosate in different mouse strains and appropriate historical control (HC) data from the performing laboratory if available

Study, Strain		Males				Females			
Wood et al, 2009, ASB2012-11492 Crl:CD-1 (ICR) BR	Dose (ppm)	0	500	1500	5000	0	500	1500	5000
	Affected	0/51	1/51	2/51	5/51	11/51	8/51	10/51	11/51
Kumar, 2001, ASB2012-11491 HsdOLA:MF1 (Swiss albino)	Dose (ppm)	0	100	1000	10000	0	100	1000	10000
	Affected	10/50	15/50	16/50	19/50*	18/50	20/50	19/50	25/50*
	HC	Study range: 6–30% Study mean: 18.4% Basis: 250 male mice in 5 studies (1996-1999 covering the in-life phase of the actual study)				Study range: 14–58% Study mean: 41.6% Basis: 250 female mice in 5 studies (1996-1999)			
Sugimoto, 1997, ASB2012-11493 Crj:CD-1 (ICR)	Dose (ppm)	0	1600	8000	40000	0	1600	8000	40000
	Affected	2/50	2/50	0/50	6/50	6/50	4/50	8/50	7/50
	HC	Study range: 3.85–19.23% Study mean: 6.33% Basis: 458 male mice in 12 studies (1993-1998)				Study range: 7.84–26.92% Study mean: 15.03% Basis: 459 female mice in 12 studies (1993-1998)			
Atkinson et al., 1993, TOX9552382, CD-1 (not further specified)	Dose (mg/kg bw/d)	0	100	300	1000	0	100	300	1000
	Affected[#]	4/50	2/50	1/50	6/50	14/50	12/50	9/50	13/50

* increase statistically significant according to original study report, for females based on percentage and not on total number of affected mice

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

Obviously, the carcinogenicity study in Swiss albino mice by Kumar (2001, ASB2012-11491) revealed an increase in malignant lymphoma incidence over the control at the top dose level of around 1460 mg/kg bw/day in both sexes but the background (control) incidence was also quite high. In fact, at least in males, the number of affected animals in the control groups was markedly higher in this strain than in three studies in CD-1 mice. It must be emphasised that this tumour is quite common in ageing mice and that Swiss mice are frequently affected (for details, see below). In this study, malignant lymphoma accounted for 54.6% of the total number of tumours when all groups are considered together.

In the most recent study in CD-1 mice by Wood et al. (2009, ASB2012-11490), there was a higher incidence of the same tumour type in high dose males (5/51 vs. 0/51 in the control group). Likewise, in the study by Sugimoto (1997, ASB2012-11493), there were a higher number of male mice affected at the exaggerated dose level of 40000 ppm (approx. 4350 mg/kg bw/day) than in the control group (6/50 vs. 2/50). In the study by Atkinson et al. (1993, TOX9552382), in contrast, there was no dose response and the incidence in the control group was similar to that at the top dose level.

In the earliest study in CD-1 mice by Knezevich and Hogan (1983, TOX9552381), malignant lymphoma was not mentioned as a separate entity but malignant lymphoblastic tumours of the lymphoreticular system in male mice did not show an increase with dose (Table 33) even though the maximum mean daily dose of 4841 mg/kg bw/day was higher than in any other study.

Table 32: Lymphoreticular neoplasia in male CD-1 mice in the study by Knezevich and Hogan (1983, TOX9552381)

Tumour type / dose (ppm	**Males**			
	0	**1000**	**5000**	**30000**
Lymphoblastic lymphosarcoma with leukaemia	1	4	3	2
Lymphoblastic lymphosarcoma without leukaemia	0	1	0	0
Composite lymphosarcoma	1	0	1	0
Lymphoreticular neoplasms (total)	2 / 48	5 / 59	4 / 50	2 / 49

If a more recent histopathological nomenclature would have been used, malignant lymphoma was covered by this data.

The data on malignant lymphoma became subject to statistical re-evaluation by means of different methods. It must be emphasised that in the first evaluation by the DS in 2013 only the statistical evaluation by the study authors according to the original study plans had been taken into account resulting in a weak but significant increase in this tumour type in high dose males and females in the study in Swiss mice but not in CD-1 mice as given in Table 31.

- For the study by Kumar (2001, ASB2012-11491), a significantly increased incidence of malignant lymphoma in males and females of the high dose group was mentioned in the study report. For analysis, the Z-test had been employed revealing a significance level of 0.002. Interestingly, when the more usual Fisher's exact test had been used, p-values of 0.077 or even 0.225 would have been obtained and the significance lost in both sexes. The trend test also provided a p-value above the significance level of 0.05, most probably because of the high control incidence (see Table 33).

Table 33: Malignant lymphoma in Swiss albino mice (Kumar, 2001, ASB2012-11491). Fisher's exact test was used to pairwise compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	**Males on study**	**Males with malignant lymphoma**	**Females on study**	**Females with malignant lymphoma**
0	50	10	50	18
15	50	15 (0.356)	50	20 (0.837)
151	50	16 (0.254)	50	19 (1.000)
1460	50	19 (0.077)*****	50	25 (0.225)*

Dose (mg/kg bw/day)	Males on study	Males with malignant lymphoma	Females on study	Females with malignant lymphoma
Trend test (p-value)		0.0655		0.068

* The original study report indicated a statistically significant increase ($p<0.05$), using the Z-test.

- In contrast, re-analysis of the studies by Wood et al. (2009, ASB2012-11490) and Sugimoto (1997, ASB2012-11493) showed statistically significant increases with dose for male CD-1 mice in the trend test (Table 34 and Table 35) but a rather low or even "zero" incidence in the control groups might be behind this finding. For the data from the Wood et al. (2009, ASB2012-11490) study, a first pairwise comparison by Fisher's exact test suggested a borderline increase at the top dose level but statistical significance was not achieved ($p = 0.056$). This result was confirmed by the chi-square test. Also for this comparison, the very low control incidence (0/51) should be taken into consideration. No evidence of an increase in malignant lymphoma was found in females.

Table 34: Malignant lymphoma in CD-1 mice (Wood et al., 2009, ASB2012-11490). Chi square test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Males with malignant lymphoma	Females on study	Females with malignant lymphoma
0	51	0	51	11
71	51	1 (1.000)	51	8 (0.611)
234	51	2 (0.475)	51	10 (1.000)
810	51	5 (0.067)[#]	51	11 (1.000)
Trend test (p-value)		0.0037		0.3590

[#] Chi –square test was chosen in accordance to the recommendations of the statistics package used. Using Fisher's exact test, a p-value of 0.056 (two-sided) was calculated. Depending on the tool used for calculation, the two-tailed Z-test produced p-values of 0.0220, 0.0219 and 0.067.

Table 35: Malignant lymphoma in CD-1 mice (Sugimoto, 1997, ASB2012-11493). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Males with malignant lymphoma	Females on study	Females with malignant lymphoma
0	50	2	50	6
165	50	2 (1.000)	50	4 (0.741)
838	50	0 (0.495)	50	8 (0.774)
4348	50	6 (0.269)	50	7 (1.000)
Trend test (p-value)		0.0085		0.2971

No evidence of an increase in malignant lymphoma was obtained upon statistical re-evaluation for

the study by Atkinson et al. (1993, TOX9552382) confirming the prior assumption (Table 36).

Table 36: Malignant lymphoma in CD-1 mice (Atkinson et al., 1993, TOX9552382). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Males with malignant lymphoma	Females on study	Females with malignant lymphoma
0	50	4	50	14
100	50	2 (0.678)	50	12 (0.657)
300	50	1 (0.362)	50	9 (0.342)
1000	50	6 (0.741)	50	13 (1.000)
Trend test (p-value)		0.0760		0.4831

It may be concluded that the statistical significance of the suspected increase in malignant lymphoma in the various studies depends very much on the statistical method that is used for data analysis. When the trend test is applied, the studies by Wood et al. (2009, ASB2012-11490) and Sugimoto (1997, ASB2012-11493) provide evidence of an effect which was not the case when pairwise comparison was performed. In contrast, the increase in the study of Kumar (2001, ASB2012-11491) was not confirmed neither by the trend test nor by a different pairwise test than the Z-test that had been used first.

According to OECD criteria (OECD 116), significance in either kind of test (i.e., trend test or pair-wise comparison) was sufficient to reject the hypothesis of a chance event. However, statistical significance is not the only criteria to decide whether or not an increase in a certain tumour type should be assumed as treatment-related. For a firm conclusion on the likeliness of an increase in malignant lymphoma in mice due to glyphosate exposure, the biological significance of a numerically higher tumour rate, the whole database in the species and the respective strains (i.e., historical control data on the background incidence of a given tumour type) and more aspects such as dose selection and dose response must be taken into consideration.

At first, dose selection and dose response in the individual studies might be of importance. In the studies by Wood et al. (2009, ASB2012-11490) and by Atkinson et al. (1993, TOX9552382) in CD-1 mice, comparable top doses of 810 or 1000 mg/kg bw/day were administered and a similar incidence of malignant lymphoma was noted in high dose males (5/51 or 6/50, respectively). However, the control group incidences were clearly different (0/51 vs. 4/50) resulting in a positive trend test in the study by Wood et al. (2009, ASB2012-11490) only. A dose of 4348 mg/kg bw/day was actually applied in the study by Sugimoto (1997, ASB2012-11493) as a maximum. The study was also performed in CD-1 mice and the malignant lymphoma incidence of 6/50 at the top dose level was similar to what was seen in the two studies mentioned before even though the applied dose was by four to five times higher. This is surprising since a further increase would be expected if it was a treatment-related effect. These doubts are further supported by the long-term study by Knezevich and Hogan (1983, TOX9552381) in which an even still higher dose of 4841 mg/kg bw/day was fed without an increase in lymphoreticular tumours in general. Unfortunately, malignant lymphoma was not mentioned as a particular pathological entity but it can be reasonably assumed that such tumours have been reported as "lymphoreticular neoplasia".Thus, if all four studies in CD-1 mice are taken together, there is no consistent dose response.

Then, the huge variability of spontaneous incidences of malignant lymphoma in mice as suggested by historical control data must be taken into consideration. This holds true for both Swiss and CD-1 mice as well as for other strains (Wogan and Pattengale, 1984, ASB2016-889). Unfortunately, reliable historical control data on malignant lymphoma incidence from the performing laboratories are available only for two of the glyphosate studies (Sugimoto, 1997, ASB2012-11493, and Kumar, 2001, ASB2012-11491). Therefore, it is necessary to use also data from the open literature or from industry databases even though such information is usually considered less relevant.

In the study in Swiss mice by Kumar (2001, ASB2012-11491), the historical control incidence from the performing laboratory was in a very wide range from 6 to 30% in male mice (study mean 18.4%) and from 14 to 58% in females (study mean 41.6%). Thus, the actual malignant lymphoma incidence in this study of 38% in males and 50% in females was above the mean values of the (relatively small) historical control and, for males, outside the historical control range. Of course, the relevance of this data is questionable since it was based on observations in only five studies employing in total 250 untreated control animals per sex. Nonetheless, it seems well in line with information that was found in the literature providing confirmation that Swiss mice are prone to developing lymphoreticular tumours. According to older articles, control incidences in male mice of Swiss or Swiss-derived strains may reach 18–27.5% and exceed 36% in females (Sher, 1974, Z22020; Roe and Tucker, 1974, ASB2015-2534; Tucker, 1979, Z83266). In a more recent publication, Tadesse-Heath et al. (2000, ASB2015-2535) even mentioned a nearly 50% lymphoma (mostly of B cell origin) incidence in a colony of CFW Swiss mice but also emphasised the contribution of widespread infections with murine oncogenic viruses to the high but remarkably variable incidence of tumours of the lymphoreticular system in this species. This problem is known for long and was often addressed in the past in textbooks of virology or mouse pathology. Already more than 30 years ago, Wogan and Pattengale (1984, ASB2016-889) described the contradictory situation as follows: “The role of oncogenic viruses in many hematopoietic tumours in mice is well established. Virtually all spontaneous or induced lymphomas which have been studied in mice contain oncogenic viruses. It is also recognized that oncogenic viruses and chemicals can act synergistically on cells in vitro and in vivo to cause tumour formation. This can be manifested by either increased incidence, decreased latency, or both. This raises the important issue as to whether a chemical which induces lymphoma in mice requires the presence of a murine oncogenic virus. If so, perhaps the induction of this tumour in mice would not be relevant to human carcinogenic risk. However, since it is possible that many other species, including man, carry undetected oncogenic virus which may act with chemicals to increase tumour burdens, considerations of viral carcinogenesis do not totally resolve the questions concerning the significance of mouse lymphoma in safety testing, except to point out that the prevalence of oncogenic viruses in mice may make them highly susceptible to the induction of lymphoma, leukaemia, and perhaps other neoplasms.” No information is available on possible abundance of oncogenic viruses in the mouse colonies from which the animals used in the glyphosate studies were obtained. During a teleconference (TC 117) on carcinogenicity of glyphosate hold by EFSA (EFSA, 2015, ASB2015-12200), it was mentioned by an U.S. EPA observer that the Kumar (2001, ASB2012-11491) study had been excluded from U.S. EPA evaluation due to the occurrence of viral infection that could influence survival as well as tumour incidences, especially those of lymphomas. However, in the study report itself, there was no evidence of health deterioration due to suspected viral infection and, thus, the actual basis of EPA’s decision is not known.

On request of the DS, reliable historical control data was provided by the Japanese laboratory in which the study by Sugimoto (1997, ASB2012-11493) had been run. In male Crj:CD-1 (ICR) mice, incidence of malignant lymphoma in this laboratory varied very much. It ranged from 3.85% to 19.23% in the control groups from 12 studies that had been performed between 1992 and 1998 (Kitazawa, 2013, ASB2014-9146). Thus, the 12% incidence at the top dose level in the study with glyphosate was well covered by the range even though it was above the mean value of 6.33%. (In

females, control incidences in the comparison studies ranged from 7.84 to 26.92% with a mean of 15.03%.)

Unfortunately, for the study of Wood et al. (2009, ASB2012-11492), the submitted historical control data was not particularly useful for the assessment. In fact, control data from a total of nine studies were submitted (Wood, 2015, ASB2015-2531) but were of not much use because incidences in male and female mice were not reported separately and since the data were apparently from the same contract research organisation but not from the same test facility. However, the mentioned study incidences ranging from 0% up to 32% (both sexes combined) show the large variability of malignant lymphoma frequency and would, theoretically, cover all male and female groups in the studies in CD-1 mice. This assumption is supported by further historical control data for CD-1 mice collected from industry databases (Giknis and Clifford, 2005, ASB2007-5200; Anonym, 2015, ASB2015-2532) or open literature (Son and Gopinath, 2004, ASB2015-2533). According to these data collections, malignant lymphoma is quite common in CD-1 mice but the reported incidences in different CD-1 strains and among the laboratories were extremely variable. Mostly, they were higher in females than in males but even in males may reach rates between 10% and 20%. The Charles River database (Giknis and Clifford, 2005, ASB2007-5200) includes data obtained in a total of 59 studies (duration 78 to 104 weeks) in CD-1 mice. The animals were bred in four different Charles River facilities in the United States and the studies were performed in 11 laboratories in North America and Europe between 1987 and 2000. The diagnosis "malignant lymphoma" was used in 42 studies revealing study incidences ranging from a minimum of 1.45 up to a maximum of 21.67% with a total mean in all untreated animals of 4.5%. The malignant lymphoma incidences in male mice receiving the highest doses in the studies by Atkinson et al. (1993, TOX9552382), Sugimoto (1997, ASB2012-11493), and Wood et al. (2009, ASB2012-11490) accounted for not more than 12% and would fit into this range even though the mean was exceeded.

On balance, based on uncertainties with regard to partly contradictory study outcomes depending on the statistical method applied, inconsistent dose response in the individual studies, and a highly variable tumour incidence as suggested by historical control data, it is not likely that glyphosate has induced malignant lymphoma in mice. A possible role of oncogenic viruses should not be ignored. Moreover, human relevance of such an effect, if occurring only as a high-dose phenomenon as it was the case here, is considered equivocal.

Renal tumours in male mice

In the IARC evaluation (IARC, 2015, ASB2015-8421), a positive trend for renal (tubular) adenoma and carcinoma in males in the study by Knezevich & Hogan (1983, TOX9552381) was highlighted. This increase had been subject to discussion already in the 1980s when this study was evaluated for the first time by U.S. EPA. At that time, re-evaluation of the histopathological findings by a "Pathology working group (PWG)" had been requested and was performed. By the DS, the positive trend can be confirmed (Table 37) even though a pairwise comparison did not indicate a statistically significant difference to the control, neither for the adenoma nor for the carcinoma or both combined.

Table 37: Renal adenoma and carcinoma in male CD-1 mice (Knezevich and Hogan 1983, TOX9552381), based on originally reported data and re-evaluation by PWG. Fisher's exact test was used to compare each treatment group to the respective

control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	N	Original report	Re-evaluation by PWG		
		Adenoma	Adenoma	Carcinoma	Combined
0	49	0	1	0	1
157	49	0 (1.000)	0 (1.000)	0 (1.000)	0 (1.000)
814	50	1 (1.000)	0 (0.495)	1 (1.000)	1 (1.000)
4841	50	3 (0.242)	1 (1.000)	2 (0.495)	3 (0.617)
Trend test (p-value)		0.0080	0.2473	0.0370	0.0339

For a more comprehensive assessment and to provide a broader view, the incidence of renal tumours in all long-term studies in male CD-1 mice was considered (Table 38). From this overview, it becomes clear that such tumours are rare but still may also occur in untreated animals. A numerically higher incidence in adenoma was seen in the study by Sugimoto (1997, ASB2012-11493) and, again, this increase was confined to male mice receiving the highest dose. Thus, there was an increase in renal tumour incidence over the overall control level in the two studies in which extremely high dose levels of 4841 or 4348 mg/kg bw/day) had been administered. The top dose levels in the studies by Wood et al. (2009, ASB2012-11490) and by Atkinson et al. (1993, TOX9552382) were much lower and no increase in renal tumours was seen. However, it must be emphasised that the same number of animals was affected in the study by Atkinson et al. (1993, TOX9552382) in the control and low dose groups as in the study by Sugimoto (1997, ASB2012-11493) at the top dose level and that the difference to 3/50 affected mice in the study by Knezevich and Hogan (1983, TOX9552381) was only marginal. Even though no historical control data from the performing laboratories was provided, a simple comparison of the control groups in the individual studies with glyphosate suggests that renal tumours may occur in untreated control males at a similar incidence than in the groups receiving very high doses.

Table 38: Incidences of renal tubule tumours in the four available glyphosate studies in male CD-1 mice

Study	Knezevich and Hogan, 1983, TOX9552381	Atkinson et al., 1993, TOX9552382	Sugimoto, 1997, ASB2012-11493	Wood et al., 2009, ASB2012-11490
Dose levels	**0, 1000, 5000, 30000 ppm**	**0, 100, 300, 1000 mg/kg bw/d**	**0, 1600, 8000, 40000 ppm**	**0, 500, 1500, 5000 ppm**
Control	1 / 49	2[#] / 50	0 / 50	0 / 51
Low dose	0 / 49	2[#] / 50	0 / 50	0 / 51
Mid dose	1[#] / 50	0 / 50	0 / 50	0 / 51
High dose	3[##] / 50	0 / 50	2 / 50	0 / 51

[#] including one carcinoma; [##] including two carcinomas

With regard to malignancy, carcinoma were reported by the PWG when re-evaluating the study by Knezevich and Hogan (1983, TOX9552381) and also by Atkinson et al. (1993, TOX9552382). In contrast, both renal tumours found by Sugimoto (1997, ASB2012-11493) were benign. It should be kept in mind that it is difficult to discriminate between benign and malignant renal tubule tumours

and, thus, combined incidence might provide the most appropriate figure.

No renal tubule tumours were seen in female mice in any of these studies.

In order to provide a complete picture, renal tumour incidences in male mice in the study by Kumar (2001, ASB2012-11491) in Swiss mice are given in Table 39 even though this study is not being considered further since another strain was employed. In total, 3 renal tumours (described as adenoma) were observed, affecting both the mid and high dose groups. According to the original study report, all neoplasia were assessed for statistical significance by means of the Z-test which was apparently negative. A Cochran-Armitage test for trend and a Peto test were also mentioned by the study author, however, it is not clear if trend analysis has been actually performed. When the renal tumours were re-analysed by the DS, there was a positive linear trend whereas Fisher's exact test failed to indicate a significant difference. No renal tumours were seen in female Swiss albino mice and there was no evidence of concomitant kidney pathology neither in males nor in females.

Table 39: Renal tubular tumours adenoma in male Swiss mice (Kumar 2001, ASB2012-11491). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Adenoma
0	50	0
15	50	0 (1.000)
151	50	1 (1.000)
1460	50	2 (0.495)
Trend test (p-value)		0.0390

Even if not fully comparable because of the strain differences, it should be remembered that the top dose incidence of 2/50 in this study was the same as seen in CD-1 mice in the study by Atkinson et al. (1993, TOX9552382) in the control and low dose groups.

With respect to CD-1 mice, the finding in the study by Sugimoto (1997, ASB2012-11493) was also subject to statistical re-evaluation for trend by the DS revealing a positive result (Table 40), most probably due to the "zero" incidence in the control group. As to be expected because of the low number of affected mice at the top dose level, the pairwise comparison (as performed also according to the original report) did not indicate a statistically significant difference.

Table 40: Renal tubular tumours adenoma in CD-1 mice (Sugimoto, 1997, ASB2012-11493). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Adenoma
0	50	0
165	50	0 (1.000)
838	50	0 (1.000)
4348	50	2 (0.495)

Dose (mg/kg bw/day)	Males on study	Adenoma
Trend test (p-value)		0.0078

On the basis of this data, it cannot be clearly distinguished whether the small increase in a rare renal tumour in mice at exaggerated dose levels that have been applied for 2 years or at least 18 months could be attributed to glyphosate itself and its toxicity, was due to long-lasting renal excretion of large amounts of an otherwise more or less inert substance or rather a chance event. The whole database, quantitative (dose) and mechanistic considerations as well as historical control data should be taken into account.

It must be emphasised that a higher number of male CD-1 mice bearing renal tumours as compared to the concurrent controls were only seen in the studies by Sugimoto et al. (1997, ASB2012-11493) and by Knezevich and Hogan (1983, TOX9552381) at the maximum doses of 4348 or even 4841 mg/kg bw/day and, therefore, cannot be either supported or contravened by the other studies in which lower maximum doses of up to 1000 mg/kg bw/day had been applied, i.e., those of Atkinson et al. (1993, TOX9552382) and Wood et al. (2009, ASB2012-11490). For the study in Swiss mice, there is no other study to match it. If increased tumour incidences are found only at the highest dose levels in a lifetime study, the occurrence of a confounding effect of excessive toxicity should be regarded very critically. Dose levels of >4000 mg/kg bw per day were well in excess of the limit dose for carcinogenicity testing (1000 mg/kg bw per day) as recommended by OECD guidance document 116. The OECD test guideline 451 for carcinogenicity studies does not give a precise recommendation but states that the highest dose level should elicit signs of minimal toxicity, with depression of body weight gain of less than 10%. However, in the studies by Sugimoto et al. (1997, ASB2012-11493) and by Knezevich and Hogan (1983, TOX9552381), however, the body weight gain in high dose males was decreased by more than 15% compared to controls. Mean terminal body weight of top dose males in the Knezevich and Hogan (1983, TOX9552381) study was by 11% lower than in the controls. In addition, there were gastrointestinal signs and lesions in the first and a significant increase in central lobular hepatocyte hypertrophy and central lobular hepatocyte necrosis suggesting some liver toxicity in the second study (see Table 30). Of particular interest was the observation of some kidney pathology in the study by Knezevich and Hogan (1983, TOX9552381). There was a positive trend for chronic interstitial necrosis in males with 12/50 affected in the high dose group versus 5/49 in the control. In females, there was a dose-related increase in proximal tubule epithelial basophilia and hypertrophy which were not seen among untreated control animals at all. Another finding in the urogenital tract in the same study was slight to mild urothelial hyperplasia in the bladder in mid and high dose males. The percentage of affected animals accounted for 6% in both the control and low dose groups but for 20% in the mid dose and for 16% in the high dose group. Even though there was no clear dose response, it may be assumed that glyphosate (acid) when administered at high doses might produce mucosal irritation. To conclude, there is some evidence that the MTD was exceeded in both studies at the highest dose level at which the number of tumour-bearing mice was slightly increased.

As outlined above in the section on mutagenicity, a genotoxic mode of action is unlikely. Occurrence of non-neoplastic lesions in the kidney was confined to an exaggerated dose level in the study by Knezevich and Hogan (1983, TOX9552381) in mice (see paragraph above) and papillary necrosis in a long-term study in male Wistar rats receiving more than 1200 mg/kg bw/day (Brammer, 2001, ASB2012-11488). On the other hand, the orally absorbed amount of ingested glyphosate is virtually completely and chemically unchanged eliminated in the urine (see section on toxicokinetics and metabolism above) and glyphosate acid is a known irritant to the eyes (see section above). However, it is questionable if irritation would sufficiently explain tumour formation in the kidney.

Historical control data from the Charles River Laboratories is available for Crl:CD1 (ICR) mice, based on 52 studies of at least 78 weeks duration that were performed between 1987 und 2000. From this data, it becomes clear that renal tumours are quite rare since adenoma were seen in five and carcinoma in four studies only. The maximum incidence for adenoma was 4% and for carcinoma 2% (Giknis and Clifford, 2005, ASB2007-5200). The top dose finding of 2/50 in the study by Sugimoto (1997, ASB2012-11493) is at the upper edge of adenoma frequency. In the study by Knezevich and Hogan (1983, TOX9552381) which is not actually covered by the timeframe of the historical database, the adenoma incidence (2%) at the top dose level would be inside the historical range whereas a carcinoma incidence of 4% was above. However, it is very difficult to distinguish between malign and benign kidney tumours and progression is frequent.

To conclude, it is not likely that the renal tumours in male mice are treatment-related for the following considerations:

- Even the incidences of affected animals at exaggerated doses exceeding the OECD-recommended limit of 1000 mg/kg bw/day and also the MTD were not statistically significantly increased when compared with the concurrent controls.
- If the whole database is taken into account, it becomes apparent that the top dose incidences in the studies by Sugimoto (1997, ASB2012-11493) and by Kumar (2001, ASB2012-11491) are the same as in the study by Atkinson et al. (1993, TOX9552382) in both the control and low dose groups and the number of affected males in the study by Knezevich and Hogan (1983, TOX9552381) was only slightly higher (3 vs. 2).
- Even the incidences at exaggerated doses are covered by the historical control range.
- No pre-neoplastic kidney lesions have been observed in treated animals.
- There is no plausible mechanism.

Haemangiosarcoma in male mice

Another tumour type was observed by Atkinson et al. (1993, TOX9552382) and highlighted by IARC. Again, the trend test was positive even though a pairwise comparison failed to indicate statistical significance. This holds true also for the study by Sugimoto (1997, ASB2012-11493) when re-evaluated by the DS (Table 41).

Table 41: Haemangiosarcoma in male CD-1 mice (Atkinson et al., 1993, TOX9552382; Sugimoto, 1997, ASB2012-11493). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	**N**	**Haemangiosarcoma**	**Dose (mg/kg bw/day)**	**N**	**Haemangiosarcoma**
Atkinson et al. (1993, TOX9552382)			**Sugimoto (1997, ASB2012-11493)**		
0	50	0	0	50	0
100	50	0 (1.000)	165	50	0 (1.000)
300	50	0 (1.000)	838	50	0 (1.000)
1000	50	4 (0.059)	4348	50	2 (0.495)
Trend test (p-value)		0.0004			0.0078

With regard to the other studies in CD1 mice, there were no haemangiosarcoma in the study by Wood et al. (2009, ASB2012-11490) in the vascular system up to the highest dose level of approx. 810 mg/kg bw/day. However, if also tumours of this type in the liver and/or kidney were taken into account, the incidence was 2/51 (control), 1/51 (71 mg/kg bw/day), 2/51 (234 mg/kg bw/day), and, again, 1/51 at the top dose level of 810 mg/kg bw/day. In the earliest study by Knezevich and Hogan (1983, TOX9552381), haemangiosarcoma was not listed as a particular histopathological entity but was observed in the spleen of one mid-dose male animal (1/50). Incidence in females, in all studies in CD-1 mice, varied between 0 and 2 but there was no dose response and the tumour occurred also in the controls (1/51 in the study by Wood et al., 2009, ASB2012-11490).

In the study by Kumar (2001, ASB2012-11491) in Swiss mice, there was no evidence of a treatment-related increase in haemangiosarcoma. This tumour type was found in one mid dose male and one control female only. Thus, this study in another strain does not need to be considered in this context.

Despite the positive trend test in two studies in CD-1 mice, this finding is not considered treatment related. According to Atkinson et al. (1993, TOX9552382), the historical control incidence in the performing laboratory ranged from 0/50 to 4/50 and, thus, would cover the incidence at the top dose level. This historical data was based on a total of six 2-year studies in CD-1 mice from the same laboratory and had been accepted by the JMPR in its 2004 evaluation of glyphosate although it was not mentioned in the study report when these studies had been performed. For the other studies with glyphosate, no historical data on haemangiosarcoma incidence in the performing laboratories is available.

Historical control data provided by Charles River indicate a very variable incidence of haemangiosarcoma. On different sites of the body, tumours of this type were seen in untreated control animals in 8 of 52 studies. The incidence varied between 1.67 and 12% (Giknis and Clifford, 2005, ASB2007-5200) covering the top dose findings in the glyphosate studies. .in mice

Furthermore, since Sugimoto (1997, ASB2012-11493) employed a more than four times higher top dose than Atkinson et al. (1993, TOX9552382), a markedly higher haemangiosarcoma incidence would have been expected if this tumour was in fact treatment-related.

Thus, there is not sufficient and convincing evidence to consider haemangiosarcoma in male mice treatment-related and sufficient for classification.

In Table 42, incidences of the three tumour types under discussion in male CD-1 mice in the four glyphosate studies are summarised with regard to dose response. This compilation allows a comparative view on all four studies in male CD-1 mice. It becomes apparent that all these tumours were present over the whole dose spectrum and in were observed in the control groups as well. No consistent increase was seen. If historical control data from the Charles River Laboratories is taken into account, all tumour incidences in all control and treated groups were below the maxima of the historical control data even though the mean values were always exceeded and, with regard to renal tumours, the top dose incidence in the study by Knezevich and Hogan (1983, TOX9552381) was at the upper boundary of the range when adenoma and carcinoma were combined.

The highest incidences were observed in groups receiving very high doses of glyphosate, i.e., 4841 mg/kg bw/day in case of renal tumours, 1000 and 4348 mg/kg bw/day in case of malignant lymphoma and 1000 mg/kg bw/day with regard to haemangiosarcoma. These dose levels were at or far above the recommended limit for testing of 1000 mg/kg bw/day. It is noteworthy that no similar or stronger increase of the latter two tumour types was seen in concurrent studies in which similar or even higher doses were administered. Concerning renal tumours, it should be acknowledged that in fact 3/50 animals were affected at a dose level of 4841 mg/kg bw/day but the number of cases in untreated controls or at a dose level of ca 100 mg/kg bw was 2/50 in another study suggesting that this tumour, even if rare, is not uncommon in male CD-1 mice. To conclude, over a wide dose range, there is no evidence of a consistent increase in any tumour type in male CD-1 mice.

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

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

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

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

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

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

4.9.2 Human information

The only source of human information on carcinogenicity of glyphosate is epidemiology. However, it is not possible to distinguish between effects of the active substance glyphosate and its co-formulants since humans are always exposed to plant protection products and their residues but hardly ever to the active substance alone. Furthermore, it is difficult if not impossible to attribute health effects including cancer to glyphosate-containing products since humans are exposed to a great number of environmental chemicals. Therefore, the actual value of such data for classification is questionable and in any case limited.

A number of epidemiological studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the scope of their conclusions regarding either pesticides in general, certain classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, few draw tenable associations with any specific cancer outcome. An essential consideration in both, risk assessment and interpreting the relevance of toxicology data, is exposure assessment. An inherent low level of confidence exists for epidemiological studies where tenuous links to exposure exist. Suggested associations between health outcomes and any possible causative agent are merely speculative if exposure cannot be confirmed and quantified.

Moreover, only a small number of cancer cases are observed in all the individual studies, making it difficult to obtain clear results. There are a lot of problems with confounders: in most studies, glyphosate is included together with several other pesticides/insecticides so that the specific effects of each individual substance are difficult if not impossible to determine with any certainty. Farmers who use one chemical substance may also use another. It is not clearly stated which formulation of glyphosate is used; that is, different brands may have been used which have slightly different chemical mixtures and co-formulants, which themselves may have carcinogenic effects. The exposure cannot be easily measured. For example, no measures from biomarkers from the blood are used. Exposure is measured through interviews or questionnaires. Here, the problem is in reliance on memory to accurately determine the amount of exposure to the chemicals. Furthermore, there may be a recall biases since individuals with cancer are more likely to think about possible reasons for their cancer than healthy individuals. Moreover, in these studies we find a problem with the classification of the cancers. Non-Hodgkin's lymphomas (NHLs) have been not consistently defined over time. The definition has changed over time due to the use of different diagnostic methods: first morphological methods, then modern immunological methods were applied. Therefore, the NHLs reported do not always comprise the same cancers. For instance, some include, others exclude hairy cell leukaemia. Multiple myelomas may also be considered presently as NHL but not previously. Some studies are thus not comparable and some comparisons are difficult because of the in- and exclusion of certain subtypes which are not the same. This may skew the picture. IARC notes in quite a number of studies that there is limited information on glyphosate exposure. On the other hand, evidence from epidemiological studies has to be considered with all necessary care since at least uncertainties due to extrapolating from animal to human toxicology is avoided in this approach.

The largest and most convincing epidemiological study of pesticide exposure and health outcomes in the United States was the Agricultural Health Study (AHS) in which glyphosate was also addressed and included. Dozens of publications have resulted from data generated in this study of approx. 57,000 enrolled farmers (applicators). Blair et al. (2009, ASB2012-11566) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was not reported to be associated with leukaemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos et al. (2005, ASB2012-11605) used data from the AHS in order to compare glyphosate use and multiple cancer endpoints. No association was noted for

glyphosate with all cancers types under investigation, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, NHL and leukaemia. In an earlier publication based on a different data set, however, De Roos et al. (2003, ASB2012-11606) had reported an association between NHL and glyphosate use. Likewise, McDuffie et al. (2001, ASB2011-364) mentioned a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. Blair et al. (2009, ASB2012-11566), in contrast, did not report an association between glyphosate use and NHL in the AHS data but a "possible association" between glyphosate use and multiple myeloma was mentioned making reference to a "suggested association" between glyphosate use and multiple myeloma suggested by De Roos et al. (2005, ASB2012-11605). However, in this paper, no significant increase in relative risk for multiple myeloma was demonstrated. Both papers by De Roos et al. will be discussed in more detail below. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009, ASB2012-11623) specifically referenced De Roos et al. (2005 ASB2012-11605) to provide no evidence of cancers of any type to be associated with glyphosate.

Lee et al. (2005, ASB2012-11882) reported a glyphosate association with gliomas, with the odds ratio differing between self-respondents (OR = 0.4) and proxy respondents (OR = 3.1). The authors expressed concern about higher positive associations observed for proxy respondents with glyphosate and several other pesticides. They suggested perhaps more accurate reporting of proxies for cases and underreporting by proxies for controls.

Monge et al. (2007, ASB2012-11909) investigated associations between parental pesticide exposures and childhood leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with "other pesticides", including paraquat, chlorothalonil and "others". No association was noted for paternal exposures, but elevated incidence of leukaemias was associated with maternal exposures to "other pesticides" during pregnancy.

Some further epidemiological studies have focused on an association between pesticide exposure and Non-Hodgkin`s Lymphoma (NHL). Hardell and Eriksson (1999, ASB2012-11838) investigated in a case-control study the incidence of NHL in relation to pesticide exposure in Sweden. 404 cases and 741 controls have been included. The authors discussed an increased risk for NHL especially for phenoxyacetic acids. Glyphosate was included in the uni-variate and multi-variate analyses. However, only 7 of 1145 subjects in the study gave exposure histories to this agent. The authors reported a moderately elevated odds ratio (OR) of 2.3 for Glyphosate. This OR was not statistically significant and was based on only 4 "exposed" cases and 3 "exposed" controls. The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43% of the pesticide use information, and the difficulty in the controlling for potential confounding factors given the small number of exposed subjects.

A further study was submitted by Hardell et al. (2002, ASB2012-11839). This study pools data from the above mentioned publication by Hardell and Eriksson (1999, ASB2012-11838) with data from a previously submitted publication from Nordström et al. (1998, TOX1999-687).

The authors found increased risks in a uni-variate analysis for subjects exposed to herbicides, insecticides, fungicides and impregnating agents. Among herbicides, significant associations were found for glyphosate and MCPA. However, in multi-variate analyses, the only significantly increased risk was found with a heterogeneous category of "other herbicides" and not for glyphosate. No information is given about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g., smoking, use of prescribed drugs etc.). In all, the above mentioned limitations of the publication of Hardell and Eriksson (1999, ASB2012-11838) are also applicable to the publication by Hardell et al. (2002, ASB2012-11839).

Fritschi et al. (2005, ASB2012-11624) submitted a case-control study with 694 cases of NHL and 694 controls in Australia. Substantial exposure to any pesticide was associated with an increase in NHL. However, no association between NHL and glyphosate can be made on the basis of this study. No information was given about exposure duration, glyphosate products used, and application rates. Therefore, the documentation is considered to be insufficient for assessment.

Eriksson et al. (2008, ASB2012-11614) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02. Results and reliability of the study are discussed below.

Alavanja et al. (2013, ASB2014-9174) reviewed studies on cancer burden among pesticide applicators and others due to pesticide exposure. In this article, the epidemiological, molecular biology, and toxicological evidence emerging from recent literature assessing the link between specific pesticides and several cancers including prostate cancer, NHL, leukaemia, multiple myeloma, and breast cancer were integrated. Glyphosate was reported to be the most commonly used conventional pesticide active ingredient worldwide. However, the only association between the use of glyphosate and cancer burden mentioned in this review was the observation of Eriksson et al. (2008, ASB2012-11614, see above).

The following epidemiological studies did not reveal an association between glyphosate and specific cancer types.

- Alavanja et al. (2003, ASB2012-11535) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.
- Multigner et al. (2008, ASB2012-11917) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong et al. (2009, ASB2012-11922).
- The lack of association between glyphosate use and prostate cancer was also supported recently in an epidemiology study in farmers in British Columbia, Canada, by Band et al. (2011, ASB2012-11555).
- Lee et al. (2004, ASB2012-11883) reported a lack of association between glyphosate use and stomach and oesophageal adenocarcinomas.
- Carreon et al. (2005, ASB2012-11585) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.
- Engel et al. (2005, ASB2012-11613) reported AHS data on breast cancer incidence among farmers' wives, with no association between breast cancer and glyphosate.
- Flower et al. (2004, ASB2012-11620) reported AHS data on parental use of specific pesticides and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.
- Andreotti et al. (2009, ASB2012-11544) reported AHS data where glyphosate was not associated with pancreatic cancer.
- Landgren et al. (2009, ASB2012-11875) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.
- Karunanayake et al. (2011, ASB2012-11865) reported a lack of association between glyphosate and Hodgkin's lymphoma.

- Pahwa et al. (2011, ASB2012-11987) reported a lack of association between glyphosate and multiple myeloma.
- Schinasi and Leon (2014, ASB2014-4819) published the results of epidemiologic research on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides. Phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL. However, no association between NHL and glyphosate was reported.
- Kachuri et al. (2013, ASB2014-8030) investigated an association between lifetime use of multiple pesticides and multiple myeloma in Canadian men. Excess risks of multiple myeloma were observed among men reported to be using other pesticides such as carbamates, phenoxy herbicides or organochlorines. However, no excess risk was observed for glyphosate.
- Cocco et al. (2014, ASB2014-7523) investigated the role of occupational exposure to agrochemicals in the aetiology of lymphoma overall, B cell lymphoma and its most prevalent subtypes. No increased CLL risk in relation to glyphosate became evident.
- Alavanja and Bonner (2012, ASB2014-9173) reviewed studies on occupational pesticide exposure and cancer risk. Twenty one pesticides identified subsequent to the last IARC review showed significant exposure-response associations in studies of specific cancers. No significant association was observed for glyphosate.
- El-Zaemey and Heyworth (2013, ASB2014-9473) reported a case control study on the association between pesticide spray drift from agricultural pesticide application areas and breast cancer in Western Australia. The findings support the hypothesis that a woman who ever noticed spray drift or who first noticed spray drift at a younger age had increased risk of breast cancer. However, it was not possible to examine whether the observed associations are related to a particular class of pesticides.
- Pahwa et al. (2011, ASB2014-9625) investigated the putative association of specific pesticides with soft-tissue sarcoma (STS). A Canadian population-based case-control study conducted in six provinces was used for this analysis. A higher incidence of STS was associated with the insecticides aldrin and diazinon after adjustment for other independent predictors. However, no statistically significant association between STS and exposure to glyphosate or other herbicides was observed.
- Koutros et al. (2011, ASB2014-9594) studied associations between pesticides and prostate cancer. No statistically significant positive association between pesticides and prostate cancer were observed. There was suggestive evidence on an increased risk (OR>1.0) with an increasing number of days of use of petroleum oil/petroleum distillate used as herbicide, terbufos, fonofos, phorate and methyl bromide. However, no increased risk was observed for glyphosate.

In a comprehensive review of the AHS publications and data, Weichenthal et al. (2010, ASB2012-12048) noted that increased rates in the following cancers were not associated with glyphosate use: overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukaemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, oesophagus cancer and thyroid cancer.

Mink et al. (2012, ASB2014-9617) submitted a comprehensive review of epidemiologic studies of glyphosate and cancer. To examine potential cancer risks in humans they reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. They also reviewed relevant methodological and biomonitoring studies of glyphosate. The review found no consistent pattern of positive associations indicating a causal relationship between

total cancer (in adults or in children) or any site-specific cancer and exposure to glyphosate.

Unfortunately, there was no overview table of epidemiologal studies in the RAR. However, more information is given in the addendum on carcinogenicity that is attached to this CLH report. The tables there were related to the evaluation of epidemiological studies by the IARC and have been copied into this CLH dossier, with few amendments, for the sake of transparency.

Table 43: Cohort studies which were considered in the IARC Monograph.

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Alavanja et al., 1996, ASB2015-7849	The Agricultural Health Study (AHS), large prospective cohort study	The only cohort study to date to have published findings on exposure and the risk of cancer at many different sites.	The data of this study were used in further studies. Conclusions are described there.	The AHS study was described in the RAR as basis for a number of publications.	Data of this publication were used for further studies. Conclusions on glyphosate are presented with these studies.
Alavanja et al., 2003, ASB2012-11535	Use of pesticides and prostate cancer risk (based on AHS)	No significant exposure-response association of glyphosate with cancer of prostate was found.	Agreement	Yes	No significantly increased risk of prostate cancer.
Andreotti et al., 2009, ASB2012-11544	Pesticide use and risk of pancreatic cancer (based on AHS)	The odds ratio for ever- versus never-exposure to glyphosate was 1.1 (0.6-1.7) while the odds ratio for the highest category of level of intensity-weighted lifetime days was 1.2 (0.6-2.6)	Agreement	Yes	No significantly increased risk of pancreatic cancer.
Blair et al., 2011, ASB2015-7868	Impact of pesticide exposure misclassification on estimates of relative risks in the AHS	Nondifferential exposure misclassification biases relative risk estimates towards the null in the AHS and tends to decrease the study power.	Glyphosate was not assessed in this study.	No, no assessment of glyphosate in this study	No assessment of glyphosate in this study
Dennis et al., 2010, ASB2015-8439	Pesticide use and risk of melanoma (based on data of AHS)	Exposure to glyphosate was not associated with cutaneous melanoma within the AHS.	Agreement	No	No increased risk of melanoma.
De Roos et al., 2005a, ASB2012-11605	Cancer incidence among glyphosate-exposed pesticide applicators (based on data of the AHS)	No increased risk of all cancers and of cancers in lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and of melanoma, all lympho-haematopoietic cancers, NHL and leukaemia. For multiple myeloma the relative risk was 1.1 (0.5-2.4) when adjusted for age, but was 2.6 (0.7-9.4), when adjusted for	Agreement with the reported results and the conclusion on limited power of the study. Further discussion of multiple myeloma in this study see also re-evaluation by Sorahan (2015,	Yes	No increased risk of all cancers and of cancers in lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and of

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
		multiple confounders. The study had limited power for the analysis of multiple myeloma. Missing data limit the interpretation of the findings.	ASB2015-2284), below		melanoma, all lympho-haematopoietic cancers, NHL and leukaemia. Interpretation of multiple myeloma is limited.
De Roos et al., 2005b, ASB2015-8437	Response in the discussion on the study of De Roos et al., 2005a, ASB2012-11605 (see above)	The study had limited power for the analysis of multiple myeloma. Missing data limit the interpretation of the findings.	Agreement	No, the paper is no study but only a response in the discussion on study of De Roos et al., 2005a, ASB2012-11605 (see above).	See De Roos et al., 2005a, ASB2012-11605
Engel et al., 2005, ASB2012-11613	Pesticide use and breast cancer risk	No difference in incidence of breast cancer for women who reported ever applying glyphosate (odds ratio 0.9 (0.7-1.1); Women who never used glyphosate but whose husband had used (no information on duration of use): odds ratio 1.3 (0.8-1.9)	Agreement	Yes	No significantly increased risk of breast cancer.
Flower et al., 2004, ASB2012-11620	Parental pesticide application and cancer risk in children; (based on data of AHS)	"For all the children of the pesticide applicators, risk was increased for all childhood cancers combined, for all lymphomas combined, and for Hodgkin lymphoma, compared with the general population." Limited power of the study for glyphosate exposure.	The cited IARC conclusion considers the risk for children of all pesticide applicators. However, this statement is not relevant for the assessment of glyphosate. There was an increased odds ratio in result of application of pesticides aldrin, dichlorvos and ethyl dipropylthiocarbamate. However, the results for glyphosate did not demonstrate any risk for childhood cancer. The odds ratios for maternal	Yes	No increased risk of childhood cancer.

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
			use and paternal use of glyphosate are even clearly below 1. Agreement with the limited power of the study.		
Landgren et al., 2009, ASB2012-11875	Pesticide exposure and risk of monoclonal gammopathy (based on data of AHS)	No association between exposure to glyphosate and risk of monoclonal gammopathy of undetermined significance, a premalignant plasma disorder that often precedes multiple myeloma; odds ratio 0.5 (0.2-1.0)	The study authors conclude a nonsignificant decrease of monoclonal gammopathy of undetermined significance (MGUS), on the large data base of the AHS.	Yes	Nonsignificant decrease of risk of MGUS which usually precedes multiple myeloma
Lee et al., 2007, ASB2015-8228	Pesticide use and risk of colorectal cancer (based on data of AHS)	Most of the 50 pesticides studied were not associated with risk of cancer of the colorectum, and the relative risks with exposure to glyphosate were 1.2 (0.9-1.6), 1.0 (0.7-1.5) and 1.6 (= 0.9-2.9) for cancers of the colorectum, colon and rectum respectively.	Agreement	No	No significantly increased risk of colorectal cancers.
Sorahan, 2015, ASB2015-2284	Glyphosate and multiple myeloma, re-analysis of AHS data; data of the study of De Roos et al., 2005a, ASB2012-11605 (see above) are reanalysed	Sorahan confirmed that the excess risk of multiple myeloma was present only in the subset with no missing information.	The author concluded that "*this secondary analysis of AHS data does not support the hypothesis that glyphosate use is a risk factor for multiple myeloma*".	No, study was published after completion of the RAR.	No significantly increased risk of multiple myeloma based on the AHS data

Table 44: Case-control studies on Non-Hodgkin lymphoma (NHL), multiple myeloma and leukaemia which were considered in the IARC Monograph.

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Brown et al., 1990, TOX2003-999	Pesticide exposure and other agricultural risk for leukaemia	The odds ratio for glyphosate was 0.9 (0.5-1.6). The study had limited power to assess effects of glyphosate.	Agreement	No, because released before 2000	No increased risk of leukaemia, limited power of the study.
Brown et al., 1993, TOX2002-1000	Pesticide exposure and multiple myeloma	The odds ratio for glyphosate was 1.7 (0.8-3.6). The study had limited power to assess effects of glyphosate.	Agreement	No, because released before 2000	Limited power of the study to assess effects of glyphosate.
Cantor et al., 1992, ASB2015-7885	Pesticides and other agricultural risk factors for non-Hodgkin lymphoma	The odds ratio for men who ever handled glyphosate was 1.1 (0.7-1.9), low power of the study to assess risk of NHL associated with glyphosate	Agreement	No, because released before 2000	No significantly increased risk of non-Hodgkin lymphoma, limited power of the study
Cocco et al., 2013, ASB2014-7523	Pesticide exposure and lymphoma risk	Odds ratio for glyphosate exposure was 3.1 (0.6-17.4); the study had a very limited power to assess the effects of glyphosate on risk of NHL	Agreement with the reported results and the conclusion on limited power of the study. Only 4 exposed cases and 2 control subjects have been considered in this study.	Yes	Very limited power of the study (only 4 exposed cases and 2 control subjects)
De Roos et al., 2003, ASB2012-11606	Pesticide exposure and risk of non-Hodgkin lymphoma	See separate assessment in this addendum	See separate assessment in this addendum	Yes	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Eriksson et al., 2008, ASB2012-11614	Pesticide exposure and risk of non-Hodgkin lymphoma	See separate assessment in this addendum	See separate assessment in this addendum	Yes	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Hardell and Eriksson, 1999, ASB2012-11838	Pesticide exposure and risk of non-Hodgkin lymphoma	The odds ratio for ever-use of glyphosate was 2.3 (0.4-13.4) in a univariate analysis, and 5.8 (0.6-54) in a multivariable analysis. The exposure frequency was low for glyphosate, and the study had limited power to detect an effect.	Agreement with the reported results and the conclusion on limited power of the study. Only 4 exposed cases and 3 control subjects have been considered in this study.	Yes	no conclusion possible because of limited power of the study (only 4 exposed cases and 3 control subjects)

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Hardell et al., 2002, ASB2012-11839	Pesticide exposure and risk of non-Hodgkin lymphoma and hairy cell leukaemia	The study is a pooled analysis of two case-control studies (see Hardell and Eriksson, 1999, TOX1999-686, ASB2012-11838 and Nordström et al., 1998, TOX1999-687 in this addendum). Increased risk was found for glyphosate only in univariate analysis (odds ratio, 3.04 (1.08-8.52)), however, the odds ration decreased in multivariate analysis to 1.85 (0.55-6.20). The exposure frequency for glyphosate was low and the study had limited power.	Agreement with the presented results and the conclusion on limited power of the study. The study is a pooled analysis of two case-control studies (see separate discussion on studies of Hardell and Eriksson, 1999, TOX1999-686, ASB2012-11838 and Nordström et al., 1998, TOX1999-687 in this addendum).	Yes	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Kachuri et al., 2013, ASB2014-8030	Pesticide exposure and risk of multiple myeloma	The odds ratio for ever-use of glyphosate was 1.19 (0.76-1.87); no association was found for light users (≤ 2 days per year, odds ratio 0.72 (0.39-1.32), the odds ratio in heavier users (>2 days per year) was 2.04 (0.98-4.23). The study had relatively low response rates.	Agreement	Yes	No increased risk of multiple myeloma for ever use of glyphosate, higher (not significant) OR if mixing or applying glyphosate >2 days per year, low response rate
Karunanayake et al., 2012, ASB2012-11865	Pesticide exposure and risk of non-Hodgkin lymphoma	Based on 38 cases exposed to glyphosate, the odds ratios were 1.14 (0.74-1.76) adjusted for age and province, and 0.99 (0.62-1.56) when additionally adjusted for medical history variables.	Agreement	Yes	No increased risk of non-Hodgkin lymphoma
Lee et al., 2004a, ASB2015-8238	Pesticide exposure and risk of non-Hodgkin Lymphoma among asthmatics	Subject with a history of asthma had a non-significantly lower risk of NHL than non-asthmatics. The odds ratio associated with glyphosate use was 1.4 (0.98-21.) among non-asthmatics and 1.2 (0.4-3.3) among asthmatics.	Agreement	No	No significantly increased risk of non-Hodgkin lymphoma for asthmatics and non-asthmatics; non-significantly lower risk of NHL for asthmatics than non-asthmatics

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
McDuffie et al., 2001, ASB2011-364	Pesticide exposure and risk of non-Hodgkin lymphoma	Odds ratio of 1.26 (0.87-1.80) and 1.20 (0.83-1.74, adjusted for age, province, high-risk exposures) were observed for exposure to glyphosate. In an analysis by frequency of exposure to glyphosate, participants with 2+ days of exposure per year had an odds ratio of 2.12 (1.2-3.73) compared with those with some but ≤ 2 days of exposure. The study was large, but had relatively low participation rates.	See separate assessment in this addendum	Yes	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Nordström et al., 1998, TOX1999-687	Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia	An age-adjusted odds ratio of 3.1 (0.8-12) was observed for exposure of glyphosate. However, the study had limited power, only 4 exposed cases and there was no adjustment for other exposures.	Agreement with reported results and conclusions on limited power, only 4 exposed cases and 5 exposed controls are considered in this study	Yes	Limited power of the study (only 4 exposed cases and 5 exposed controls)
Orsi et al., 2009, ASB2012-11985	Pesticide exposure and risk of lymphoid neoplasms	The odds ratios associated with any exposure to glyphosate were 1.2 (0.6-2.1) for all lymphoid neoplasms, 1.0 (0.5-2.2) for NHL, 0.6 (0.2-2.1) for lymphoproliferative syndrome, 2.4 (0.8-7.3) for multiple myeloma, and 1.7 (0.6-5.0) for Hodgkin lymphoma.	Agreement with reported results. It should be considered in the discussion on an association between glyphosate and NHL that the OR of NHL in this study (12 exposed cases and 24 exposed controls) was 1.0.	No	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Waddell et al., 2001, ASB2015-8037	Use of organophosphate pesticides and risk of non-Hodgkin lymphoma	IARC compared the numbers of cases and controls in this study with the study of De Roos et al., 2003; however, no information on glyphosate in this study	No information on glyphosate	No, no information on glyphosate	no information on glyphosate
Zahm et al., 1990, ASB2013-11501	Exposure to 2,4-D and risk of non-Hodgkin Lymphoma	The study was mentioned by IARC because data were used in the study of De Roos et al., 2003	No information on glyphosate	No, no information on glyphosate	no information on glyphosate

Table 45: Case-control studies on other cancer types and meta-analyses which were considered in the IARC Monograph.

Study (Author/year)	Subject	Evaluation IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Lee et al., 2004b, ASB2012-11883	Pesticide use and risk of adenocarcinomas of stomach and oesophagus	For ever use of glyphosate, the odds ratio was 0.8 (0.4 - 1.4) for cancer of the stomach, and 0.7 (0.3 - 1.4) for oesophageal cancer; the power of the study was limited.	Agreement	Yes	No increased risk of adenocarcinomas of stomach and oesophagus
Ruder et al., 2004, ASB2015-8078	Pesticide exposure and risk of gliomas	No association was found with any of the pesticides assessed, including glyphosate. Glyphosate use was assessed, but specific results were not presented.	Agreement	No	No increased risk of gliomas
Carreon et al., 2005, ASB2012-11585	Pesticide exposure and risk of gliomas	There was a reduced risk for glyphosate (OR 0.7 (0.4 - 1.3).	Agreement	Yes	Reduced risk of gliomas
Lee et al., 2005, ASB2012-11882	Pesticide use and risk of gliomas	There was a non-significant excess risk with glyphosate use for the overall group, but there was inconsistency between observations for self-responds and observations for proxy respondents. The study had limited power to detect an effect of glyphosate use and was difficult to interpret.	Agreement	Yes	Limited power of the study, difficult to interpret
Pahwa et al., 2011, ASB2014-9625	Pesticide exposure and risk of soft-tissue sarcoma	The fully adjusted odds ratio for glyphosate was 0.90 (0.58 - 1.40).	Agreement	Yes	No increased risk of soft-tissue sarcoma
Monge et al., 2007, ASB2012-11909	Pesticide exposure and risk of childhood leukaemia	Association of childhood cancer with glyphosate were reported only for an “other pesticides” category that also included other chemicals, glyphosate was not specifically assessed.	Agreement	Yes	No specific assessment of glyphosate
Schinasi and Leon, 2014, ASB2014-4819	Meta-analysis, exposure to pesticides and non-Hodgkin lymphoma	The meta-analysis for glyphosate included six studies and yielded a meta-risk ratio of 1.5 (1.1 - 2.0). The working group noted that the most fully adjusted risk estimates from the	Agreement, see separate assessment in this addendum (section 2.4).	Yes	See separate assessment in this addendum (section 2.4).

Study (Author/year)	Subject	Evaluation IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
		articles by Hardell et al. (2002, ASB2012-11839) and Eriksson et al. (2008, ASB2012-11614) were not used in this analysis. After considering the adjusted estimates of the two Swedish studies in the meta-analysis, the Working Group estimated a meta-risk-ratio of 1.3 (1.03 - 1.65).			

OR, odds ratio

4.9.3 Other relevant information

In the IARC Monograph, oxidative stress was discussed as a possible mechanism of carcinogenicity. For detailed mechanistic information on e.g. oxidative stress please refer to the addendum to the RAR or to the RAR, that are both attached to this CLH report. However, with regard to oxidative stress it was concluded in the addendum that from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for the active substance glyphosate and glyphosate based formulations.

4.9.4 Summary and discussion of carcinogenicity

For glyphosate, a large quantity of animal data regarding carcinogenicity was submitted by different applicants and is partly also available from published scientific literature. At least six acceptable chronic toxicity and carcinogenicity studies in rats and five carcinogenicity studies in mice have been evaluated. Therefore, all available data were considered together using a weight of evidence approach with consideration of the biological significance, dose response, relationship of the highest doses used to the maximum tolerated dose and the consistency of the neoplastic findings among the studies.

In the rat, no evidence of carcinogenic effects was evident and only occasional increases in few different tumour types (pancreas, liver, thyroid, and testes) were observed in two older studies which one is considered not acceptable any longer if current standards are applied. These findings were not confirmed in five more recent, guideline-compliant studies employing very high dose levels. Moreover, the pancreatic tumours did not show a dose response. When the whole toxicological profile of glyphosate is taken into consideration, the pancreas, the thyroid and the testes were no target organs of this substance and liver effects of glyphosate were very limited. The overall conclusion can be drawn that glyphosate was not carcinogenic to the rat.

In the mouse, the incidences in malignant lymphoma, in renal tumours and haemangiosarcoma in male animals were considered in detail. Slightly higher incidences when compared with concurrent controls were confined to very high dose levels above the OECD-recommended limit dose of 1000 mg/kg bw/day and exceeding the MTD. In addition, the outcome of statistical tests was contradictory. Mostly, but not always, trend tests revealed statistical significance but pairwise comparisons failed to detect a significant difference relative to the control group. The reported incidences of all three tumour types fell within their historical control range which were, however, of variable reliability. If the four studies in CD-1 mice are considered together, it becomes apparent that all tumours were observed also in the control groups and in some groups receiving lower doses in at least one concurrent study. Furthermore, the results were not consistent with regard to dose responses. To conclude, there is not enough evidence to consider the tumours in mice as treatment-related.
Epidemiological studies revealed partly contradictory results. However, in most studies, no association with an exposure to glyphosate could be established. In particular, the largest study, i.e., the AHS (see above), was negative. Taken together, the epidemiological data does not provide convincing evidence that glyphosate exposure in humans might be related to any cancer type. 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. Thus, the results of the studies are associated to different formulations containing glyphosate or mixtures of different active substances.

4.9.5 Comparison with criteria

The following criteria for classification as a carcinogen are given in CLP regulation:

CLP regulation

A substance is classified in Category 1 (known or presumed human carcinogens) for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:

— human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or
— animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).

In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

The placing of a substance in Category 2 (suspected human carcinogens) is done on the basis of 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 (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.
[…]
3.6.2.2.3. Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms ‘sufficient’ and ‘limited’ have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows:

(a) Carcinogenicity in humans
The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

— sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;
— limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

(b) Carcinogenicity in experimental animals
Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals.
The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

— sufficient evidence of carcinogenicity: 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;
— limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

CLP regulation
3.6.2.2.4. Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here. 3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner. 3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are: (a) tumour type and background incidence; (b) multi-site responses; (c) progression of lesions to malignancy; (d) reduced tumour latency; (e) whether responses are in single or both sexes; (f) whether responses are in a single species or several species; (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; (h) routes of exposure; (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; (j) the possibility of a confounding effect of excessive toxicity at test doses; (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects.

General remark: For the majority of chemical substances evaluated under the CLP-Regulation, normally one study addressing each endpoint is required and usually sufficient for classification and labelling purposes. In contrast, for glyphosate, a large quantity of animal data regarding carcinogenicity was submitted by different applicants and at least six acceptable chronic toxicity and carcinogenicity studies in rats and five carcinogenicity studies in mice have been evaluated. In such a situation, the criteria of the CLP-Regulation may not be applicable directly to the available information for glyphosate. Instead, all available data should be considered together using a weight of evidence approach with consideration of the biological significance, relationship of the applied doses to the maximum tolerated dose and the consistency of the neoplastic findings. Basing any conclusion only on the statistical significance of an increased tumour incidence identified in a single study should be avoided.

Category 1A is not applicable since epidemiological studies do not suggest a strong link of glyphosate exposure to human cancer. In most studies, including the by far largest one, no association could be established. The DS concluded in accordance with IARC (2015) „*There is limited evidence in humans for the carcinogenicity of glyphosate.*" This is perhaps the best description of the available data since the other IARC categories ("*Evidence suggesting lack of carcinogenicity*"; "*Inadequate evidence of carcinogenicity*"; "*Sufficient evidence of carcinogenicity*") are even less suitable.

Category 1B is also not applicable since experimental evidence in laboratory animals is far from being "sufficient". Furthermore, the active substance glyphosate is devoid of genotoxic potential.

In the rat, tumours were only occasionally seen. For pancreatic tumours, no dose response became apparent in the two studies in which an increase was observed (Lankas, 1981, TOX2000-595, TOX2000-1997; Stout and Ruecker 1990, TOX9300244). Moreover, these tumours could not be

reproduced in any other long-term study. The same holds true for liver and thyroid tumours that were found in one and the same study (Stout and Ruecker 1990, TOX9300244) at the highest dose level. For a substance such as glyphosate for which a large number of independent studies is available, reproducibility is crucial. An increase in testicular tumours in an old and rather deficient study (Lankas, 1981, TOX2000-595, TOX2000-1997) was clearly a chance event since they occurred at a relatively low dose level but were not seen in six other valid studies in which much higher doses were administered. Thus, carcinogenicity to rats can be excluded with a high degree of certainty.

In the mouse, the situation is slightly different and three tumour types were considered in detail.

First, the slightly higher incidences in the rather common malignant lymphoma in three studies (Sugimoto, 1997, ASB2012-11493; Kumar, 2001, ASB2012-11491; Wood et al., 2009, ASB2012-11490) were not considered to be treatment-related when a weight of evidence approach was taken. The very different dose levels in all the studies and the dose-specific incidences were included as well as the high variability in spontaneous occurrence of this tumour type and also the statistical uncertainties.

Renal tumour incidences and haemangiosarcoma incidences in male mice from three or two out of five studies, respectively, were slightly higher when compared to concurrent controls at very high dose levels at or exceeding the OECD-recommended limit of 1000 mg/kg bw/day and sometimes being above the MTD. Statistical significance was only observed with a trend test but not in pair-wise tests. Furthermore, the low incidences even at high doses fell within the historical control ranges and the findings were not consistent among the acceptable studies in mice. Thus, these findings were considered not of relevance for assessment of carcinogenicity.

Category 2 is also not applicable based on haemangiosarcoma incidences and the respective dose response considerations. In addition to being in the historical control range, this tumour type was also seen in the control and treated groups in other studies with glyphosate (Kumar, 2001, ASB2012-11491; Wood et al., 2009, ASB2012-11490), without evidence of a dose response relationship. The difference between these figures and the incidence at the top dose levels in two studies (Atkinson et al., 1993, TOX9552382; Sugimoto, 1997, ASB2012-11493) is small or missing (1 or 2 vs. 4 and 2; see Table 42). Statistical significance with the trend test may be explained by the zero incidence in concurrent controls in the studies by Atkinson et al. (1993, TOX9552382) or Sugimoto (1997, ASB2012-11493). Furthermore, there was no increase in the Sugimoto study even though the dose level was by more than four times higher than applied by Atkinson et al. (1993, TOX9552382).

With regard to the incidences in kidney tumours in the studies by Knezevich and Hogan (1983, TOX9552381) and Sugimoto (1997, ASB2012-11493) at the top dose level, it should be noticed, on one hand, that the MTD was exceeded and, on the other hand, that a similar incidence of renal tumours (2 vs. 3 or 2) had been seen in the study by Atkinson et al. (1993, TOX9552382) in both the control and low dose group (see Table 42). Furthermore, no pre-neoplastic kidney lesions have been observed in treated animals, even at excessive dose levels. Thus, also for this tumour type, there is no convincing evidence that it is related to glyphosate administration.

On balance, this inconsistent data is not sufficient for classification and labelling of glyphosate as a category 2 carcinogen.

Based on the available data no mode of action could be identified. Mechanistic data, e.g., providing evidence of oxidative stress are partly contradictory but should not be given much weight in a situation where a very comprehensive database of high quality long-term studies in laboratory animals is available.

4.9.6 Conclusions on classification and labelling

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.

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 *et al*., 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 *et al*., 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 *et al*., 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%); 0/57		2/54 (3.7%); 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 *et al*., 1993	2# / 50 (4%)	2# / 50 (4%) (100 mg/kg	0 / 50 (300 mg/kg	0 / 50 (1000 mg/kg	No significant increas

CD-1		bw/d)	bw/d)	bw/d)	
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 *et al*., 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
Kumar *et al*., 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

[a]PWG 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 *et al*., 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	

Hemangiosarcomas are vascular tumours and they were mostly found in liver and spleen. Increased incidences of haemangiocarcomas 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 *et al.*, 2009; Crl: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 Cochran-Armitage trend test	p=0.0037			*p= 0.056*	No significant increase			
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 Cochran-Armitage trend test	p=0.0085			p= 0.269	No significant increase			
Atkinson *et al.*, 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'exact test Cochran-Armitage trend test	p=0.076			p= 0.741	No significant increase			
Knezevich and Hogan[a] 1983; Crl: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' exact test Cochran-Armitage test	No significant increase				No significant increase			
Kumar *et al.*, 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 Cochran-Armitage trend test	p=0.065			p=0.077	p=0.068			p= 0.225

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

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/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 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

[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.

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 of mice. No significant increase in tumour incidence was seen in female mice in this study. In the second feeding study, there was a significant positive trend in the incidence of haemangiosarcoma 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** studies[2] 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 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 studies[3].

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

[2] 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 that 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.

[3] 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.

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 particpants 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.

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 ratio[4] (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

[4] 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.

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.

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, 513 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)[5]. 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)[6] 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 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,

[5] Mentioned in comment no. 161 in the public consultation.

[6] Mentioned by the DS in a reply to comment no. 126 in the public consultation.

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.

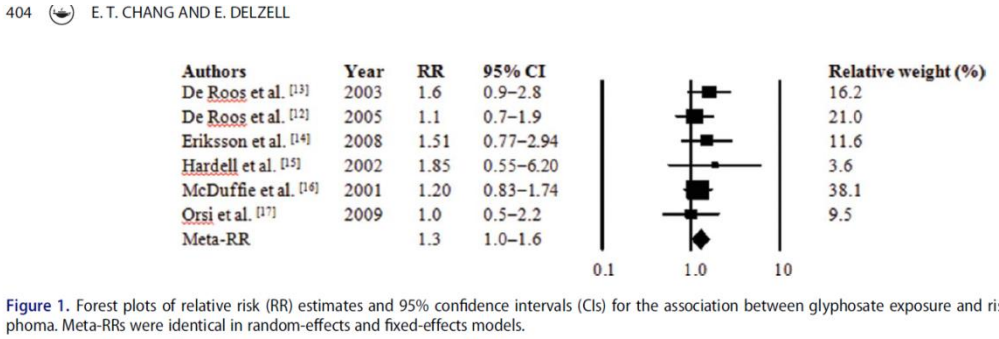

Figure from Chang and Delzell, 2016

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

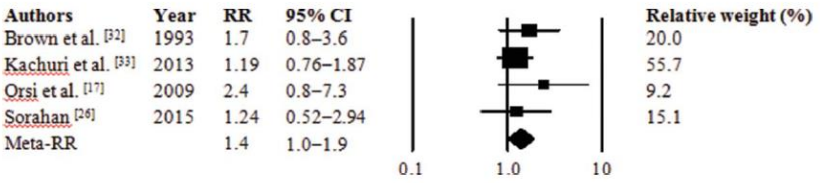

Figure 2. Forest plots of relative risk (RR) estimates and 95% confidence intervals (CIs) for the association between glyphosate exposure and risk of multiple myeloma. Meta-RRs were identical in random-effects and fixed-effects models.

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. Immunosupression 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.

Supplemental information - In depth analyses by RAC

Analyses

Table: Overview of the relationship between the most relevant epidemiological studies and analyses to assess NHL and MM from glyphosate based herbicide in humans:

Study type	Population	Study	Study	Included in meta-analysi	Included in meta-analysi	Included in meta-analysi	Included in re-analy	Included in paper by	Included in review by	Included in review by

				s by Chang and Delzell (2016)	**s by Schinasi and Leon (2014)**	**s by IARC monograph (2015)**	**sis by Sorahan (2015)**	**Alavanja et al. (2013)**	**Mink et al. (2012)**	**Weichenthal et al. (2010)**
Prospective cohort study	AHS	De Roos *et al*. (2005)		X (NHL)	X (NHL)	X (NHL)	X (MM)		X (NHL)	X (NHL) X(MM)
Re-analysis	AHS	Re-analysis of AHS data reported by De Roos *et al*. (2005)	Sorahan (2015)	X (MM)						
Population-based case-control study	Sweden	Hardell and Eriksson, 1999								
Population-based case-control study. Pooled analysis of Hardell and Eriksson 1999 and Nordstrom *et al*., 1998	Sweden	Hardell *et al*., 2002		X (NHL)	X (NHL)	X (NHL)			X (NHL)	
Population-based case-control study	Sweden	Eriksson *et al*., 2008		X (NHL) and B-cell lymphoma)	X (NHL, and B-cell lymphoma)	X (NHL)		X (NHL)	X (NHL)	
Included in Hardell *et al*., 2002	Sweden	Hardell and Eriksson 1999							X (NHL)	
Included in Hardell *et al*., 2002	Sweden	Nordstrom *et al*., 1998							X (NHL)	
Population-based case-control study	Canada	McDuffie *et al*., 2001		X (NHL)	X (NHL)	X (NHL)		X (NHL)	X (NHL)	

	Canada	Pahwa *et al*., 2012								
Population-based case-control study	Canada	Kachuri *et al*., 2013		X (MM)						
Pooled data analysis from three case-controls studies (Cantor1992; Hoar1986; Zahm1990)	Midwestern United States (Iowa and Minnesota, Kansas, Nebraska	De Roos *et al*., 2003		X (NHL)	X (NHL)	X (NHL)			X (NHL)	
Population-based case-control study (included in De Roos *et al*. 2003)		Cantor *et al*. (1992)							X (NHL)	
Population-based case-control study	Iowa	Brown *et al*., 1993		X (MM)						
Population-based case-control study	AHS	Lee *et al*. 2004 a;b							X (NHL)	X (NHL) X(MM)
Hospital-based case-control study	France	Orsi *et al*., 2009		X (NHL and MM)	X (NHL)	X (NHL)				
European multi-center case-control study	6 European countries (ES, FR, DE, IE, IT, CZ)	Cocco *et al*., 2013	Epilymph	X (subtype B-cell NHL)	X (subtype B-cell NHL)					

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

4.10.1.1 Non-human information

The reproductive toxicity of glyphosate was tested in a large number of two-generation studies in rats of which 6 may be considered fully valid or at least supplementary from a current point of view. These studies are summarised in Table 46, along with a (deficient) three-generation study.

The DS is aware of three further reproduction studies which have been referred to in an older EU evaluation (Germany, 1998, ASB2010-10302). No adverse effects were reported in any of these studies but they are not considered to be suitable for the purpose of classification and labelling. In three-generation studies by Schroeder and Hogan (1981, TOX9552385) and by Bhide (1988a, TOX9551965), the top dose levels of 30 or approx. 15 mg/kg bw/day were much too low and could not be expected to reveal any toxic effect. The same holds true for a non-guideline "segment I" study with gavage administration of up to 10 mg/kg bw/day by Bhide (1988b, TOX9551832). A published reproduction study (Dallegrave et al., 2007; ASB2012-2721) was performed with a commercial formulation and, thus, is also not useful for classification and labelling of the active substance.

Table 46: Reproductive (two-generation) studies with glyphosate in rats

Reference; Study identification; Purity; Owner	Study type, strain, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
Dhinsa et al., 2007; ASB2012-11494; 95.7%; Nufarm	Two-gen., Sprague-Dawley, diet	0, 1500, 5000, 15000 ppm	Parental, reproductive, offspring: 5000 ppm (351 mg/kg bw/d)	Parental, reproductive, offspring: 15000 ppm (1000-1600 mg/kg bw/d)	Parental.: liver, kidney wt↑; Repro: homogenisation resistant spermatid count↓; Off-spring: delay in preputial separation in F1 males
Moxon, 2000; TOX2000-2000; 97.6%; Syngenta	Two-gen., Wistar-derived AlpK, diet	0, 1000, 3000, 10000 ppm	Parental, offspring: 3000 ppm (293 mg/kg bw/d); Reproductive: 10000 ppm (985 mg/kg bw/d)	Parental, offspring: 10000 ppm (985 mg/kg bw/d); Reproductive: not established	Parental, offspring: bw↓ (F1 pups & F1-adults)
Takahashi, 1997; ASB2012-11495; 94.61%; Arysta	Two-gen., Sprague-Dawley, diet	0, 1200, 6000, 30000 ppm	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*; TOX9300009; 96.8%; ADAMA	Two-gen., Wistar rat, diet	0, 10, 100, 1000, 10000 ppm	Parental, offspring & reproductive 10000 ppm (700-800 mg/kg bw/d)	-	No treatment related effects

Reference; Study identification; Purity; Owner	Study type, strain, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
Brooker et al., 1992**; TOX9552389; 99.2%; Cheminova	Two-gen., Sprague-Dawley, diet	0, 1000, 3000, 10000 ppm	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; TOX9552387; 97.67%; Monsanto	Two-gen., Sprague – Dawley rat, diet	0, 2000, 10000, 30000 ppm	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↓
Antal, 1985***; Alkaloida	Three-gen., CD rat, diet	0, 200, 1000, 5000 ppm	Parental, offspring & reproductive: 5000 ppm (462-502 mg/kg bw/d)	-	No treatment related effects

*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, TOX9552388) also available but without impact on classification and labelling (see attached RAR)
***study not valid according to current standards because of major reporting deficiencies

It should be explained here that the "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, TOX9552387) were affected. Clinical signs or macroscopic findings were also reported when occurring in a higher number of animals as in the control group but were not always subject to statistical evaluation or did not gain statistical significance in all cases. Not all of the mentioned findings were observed necessarily at the LOAEL but sometimes only at higher dose levels. In any case, statistical significance was taken into account when the NOAELs/LOAELs in the individual studies were established.

Parental toxicity was confined to minor effects at high dose levels only. Sometimes, the findings were not consistent among the studies. The cellular alterations in parotid (males and females) and submaxillary (females only) salivary glands in F0 and F1 animals as known before from subchronic and long-term studies were reported only by Brooker et al. (1992) and in the preceding range-finding experiment but were presumably not investigated in the other studies. In addition to these histological findings, high dose (approx. 670 mg/kg bw/day) parental effects comprised gastrointestinal disturbances and a decrease in body weight whereas food and water consumption were increased.

Dhinsa et al. (2007, ASB2012-11494) observed higher absolute and relative organ weights of the liver (F0 & F1 females) and the kidneys (F0 females) at the highest dose level of 15000 ppm (1000 – 1600 mg/kg bw/day). The same effect on organ weights had been reported by Takahashi (1997) in F0 and F1 animals of both sexes, along with decreased prostate weight (F1), loose stool (F0/F1, both sexes), reduced body weight (F0/F1 males) and caecum distention (F0/F1, both sexes). All these findings, however, were confined to an exaggerated dose of 30000 ppm (>2000 mg/kg bw/day). At the same, very high dietary dose, a reduction in body weight gain and gastrointestinal effects (soft stool) had been described in adult animals in the earliest reproduction study by Reyna (1990, TOX9552387).

No evidence of reproductive toxicity was observed in any of these studies apart from a rather equivocal reduction in litter size in the study by Reyna (1990, TOX9552387) at a dose level of more than 2000 mg/kg bw/day. In the two litters produced by the F0 generation, a non-significant reduction by up to 10% was observed which was less pronounced in the F1. This dose is far above any limit dose and, furthermore, a lower litter size was not confirmed in the study by Takahashi (1997, ASB2012-11495) in which the same dietary concentration of 30000 ppm had been tested. A decrease in homogenisation resistant spermatids in the Cauda epididymidis has been observed by Dhinsa et al. (2007, ASB2012-11494) after administration of 15000 ppm but had no impact on fertility or reproductive success and, thus, was of questionable relevance. This reduction (Control: 399.9 million/gram; 15000 ppm: 309.0 million/gram) was noted in F0 males but was not reproducible at any dose levels in F1 males.

Weak 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. In addition, a significant delay in sexual maturation in male pups (F1) became apparent at the top dose level of 15000 ppm (~1000 mg/kg bw/day) in the study by Dhinsa et al. (2007, ASB2012-11494) because preputial separation was delayed, occurring after 45.9 days on average versus 43.0 days in the control group. At attainment of sexual maturation as indicated by preputial separation, the mean bodyweight of the male pups was 230 g as compared to 210 g in the control group. This effect was not related to a decrease in the bodyweight and bodyweight gain of the male pups (followed up to day 21). A treatment-related effect on the sexual development of male offspring cannot be excluded although this later onset of sexual maturation had no impact on subsequent reproductive performance. It is important to note that this finding occurred at the limit dose at which parental toxicity was also apparent. Furthermore, it was not confirmed in any of the other reproduction studies.

In summary, rigorous testing of glyphosate up to very high doses in a number of comprehensive studies did not provide evidence of reproductive or offspring toxicity. The few observed effects were small, of equivocal relevance and confined to parentally toxic dose levels. There is no need for classification for effects on sexual function and fertility, based on the animal studies.

4.10.1.2 Human information

Several epidemiological studies are available in which a possible impact of glyphosate exposure on reproductive outcome was investigated. Parameters under study comprised fecundity, miscarriage, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, or the occurrence of attention-deficit disorder / attention-deficit hyperactive disorder (ADD/ADHD) in children. In most instances, glyphosate and reproductive outcomes lack a statistically significant positive association, as described in a recent review of glyphosate non-cancer endpoint publications (Mink et al., 2011, ASB2012-11904). For ADD/ADHD, a positive association with glyphosate use had been claimed by Garry et al. (2002, ASB2012-11626) but the reported incidence of approx. 1 % in the study population was well below the general population incidence rate of approx. 7 %.

For more information, see Vol. 3 of the attached RAR.

In general, the relevance of epidemiological data to detect effects of glyphosate on fertility or reproductive performance is quite limited. This is mainly due to the fact that operators, bystanders, or residents are exposed to plant protection products containing glyphosate but not to the active substance itself. Furthermore, there is always mixed exposure to a variety of chemicals in the environment or to their residues in our diet. The extent of exposure is mostly unknown.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

The developmental toxicity and teratogenicity of glyphosate were tested in a great number of studies in rats and rabbits.

Rat

The available valid (guideline-compliant) developmental studies in rats are summarised in Table 47 whereas the few published studies are briefly mentioned below.

Table 47: Developmental toxicity studies in rats

Reference; Study identification; Purity; Owner	Strain, route, duration of treatment	Dose levels	NOAEL	LOAEL	Targets / Main effects
Moxon, 1996; ASB2012-10080; 95.6%; Syngenta	Alpk (Wistar derived), gavage, d 7-16 p.c.	0, 250, 500, 1000 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Not applicable	None
Hatakenaka, 1995 ASB2012-11497; 95.68%; Arysta	CD (SD), gavage, d 6-15 p.c.	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↑
Brooker et al., 1991, TOX9552393; 98.6%; Cheminova	CD, gavage, d 6-15 p.c.	0, 300, 1000, 3500 mg/kg bw/d	Maternal & developmental: 300 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Maternal: slight bw gain↓, noisy respiration (2/25); Development: ossification↓, skeletal anomalies
Suresh, 1991, TOX9551105; 96.8%; ADAMA	Wistar, gavage, d 6-15 p.c.	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; TOX9552392; 98.7%; Monsanto	Charles River, gavage, d 6-19 p.c.	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, diarrhea; Development: bw↓, post-implantation losses
Anonym (Author perhaps Antal), 1981; TOX9650160; purity 96.8%; Alkaloida	CFY, diet, d 6-18 p.c.	Calculated to be 0, 22, 103, 544 mg/kg bw/d	Maternal & developmental: 544 mg/kg bw/d	Not applicable	None

It should be explained here that the “main effects” were statistically significant if body weight and organ weights or developmental parameters were affected. Clinical signs were also reported when occurring in a higher number of animals as in the control group but were not always subject to statistical evaluation or did not gain statistical significance in all cases. Not all of the mentioned findings were observed necessarily at the LOAEL but sometimes only at higher dose levels. In any case, statistical significance was taken into account when the NOAELs/LOAELs in the individual

studies were established. The same holds true for the studies in rabbits addressed below.

More recently, a developmental toxicity study in outbred Wistar-RIZ rats was published by Chruścielska et al. (2000b, ASB2013-9831). Glyphosate (source and purity not given) was administered to 20 pregnant females per group by oral gavage from day 7 through day 14 of pregnancy at dose levels of 750, 1500 or 3000 mg/kg bw/day. No evidence of maternal or developmental toxicity was observed but reporting of this study was so brief that its quality cannot be assessed.

A further developmental study in Wistar rats was performed by Bhide (1986, TOX9551834) in which no signs of maternal or developmental toxicity were observed up to the highest dose level of 500 mg/kg bw/day but that study was flawed by many deficiencies putting its validity and reliability into question.

Another published developmental study (Dallegrave et al., 2003, ASB2012-11600) was performed with a commercial formulation and, therefore, is not suitable for classification and labelling of the active substance.

Thus, evaluation of glyphosate for a developmental toxicity and possible teratogenicity to rat foetuses is based on the six studies which are compiled in Table 43.

Severe maternal effects (mortality) were confined to the exaggerated dose of 3500 mg/kg bw/day in the study by Tasker and Rodwell (1980, TOX9552392). Up to the limit dose of 1000 mg/kg bw/day there were only rather weak effects such as gastrointestinal signs or a lower body weight gain.

Likewise, no teratogenic potential was seen in these studies. The lowest NOAEL for developmental effects was 300 mg/kg bw/day and the LOAEL was 1000 mg/kg bw/day, based on the studies by Brooker et al. (1991, TOX9552393) and Hatakenaka (1995, ASB2012-11497). In the first study, evidence of delayed ossification and increased incidence of foetuses with skeletal anomalies was observed at 1000 mg/kg bw/day whereas a slight increase in lumbar ribs (11 out of 7 litters compared to 4 out of 2 litters in control animals) was observed in the second. With regard to the single dose study by Suresh (1991, TOX9551105), it was acknowledged that a developmental NOAEL could not be established. At the same dose level, a higher incidence of delayed ossification (caudal vertebral arch, forelimb proximal & hindlimb distal phalanges) was observed and considered adverse, despite the fact that delayed ossification of other parts of the skeleton (skull) was more frequently seen in the control. However, these findings are not of concern because a robust NOAEL for developmental toxicity well below this high dose was established in the other studies.

These previously submitted studies did not show any teratogenic potential in rats. At the very high dose level of 3500 mg/kg bw/day causing maternal toxicity and in one study even mortality, post-implantation loss and both skeletal variations and retardations were observed (Brooker et al., 1991, TOX9552393; Tasker and Rodwell, 1980, TOX9552392). In the most recent study by Moxon (1996, ASB2012-10080), no effects were seen up to 1000 mg/kg bw/day, i.e., the highest dose tested.

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/day (approx. 10000 ppm) via the diet (Anonym, author perhaps Antal, 1981, TOX9650160).

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

Rabbit

For assessment of developmental toxicity of glyphosate in rabbits, seven studies by oral gavage are available of which one (Bhide and Patil, 1989, TOX9551960) is flawed by serious deficiencies and may be considered with strong reservations only. The studies are summarised in Table 48.

Table 48: Developmental toxicity studies with glyphosate in rabbits

Reference; Study identification; Purity; Owner	Strain, duration of treatment, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
Coles and Doleman, 1996; ASB2012-11499; 95.3%; Nufarm	NZW rabbit, d 7-19 p.c., gavage	0, 50, 200, 400 mg/kg bw/d	Maternal & developmental: 50 mg/kg bw/d	Maternal & developmental: 200 mg/kg bw/d	Maternal: mortality (2 deaths at top dose), bw gain↓; Development: post-implantation loss
Moxon, 1996; TOX2000-2002; 95.6%; Syngenta	NZW rabbit, d 8-20 p.c., gavage	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: food intake and bw gain ↓, clinical signs; Development: foetal wt ↓, ossification retarded
Hojo, 1995, ASB2012-11498; 97.56%; Arysta	Japanese White rabbits (Kbl:JW), d 6-18 p.c., gavage	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, abortion; Development: none
Suresh et al., 1993*; TOX9551106; 96.8%; ADAMA	NZW rabbit, d 6-18 p.c., gavage	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; Development: no clear-cut effects up to 100 mg/kg bw/d (high dose group excluded due to low number of foetuses and litters)
Brooker et al., 1991; TOX9552391; 98.6%; Cheminova	NZW rabbit, d 7-19 p.c., gavage	0, 50, 150, 450 mg/kg bw/d	Maternal: 50 mg/kg bw/d; Developmental:1 50 mg/kg bw/d	Maternal: 150 mg/kg bw/d; Developmental: 450 mg/kg bw/d	Maternal: mortality (1 at top dose), clinical signs (GI-tract), food intake and bw gain ↓; Development: late embryonic death, post implantation loss, cardiac malformations
Bhide & Patil, 1989**; TOX9551960; Lot 38, 95%; Barclay, Luxan	NZW rabbit, d 6-18 p.c., gavage	0, 125, 250, 500 mg/kg bw/d	Maternal & developmental: 250 mg/kg bw/d	Maternal & developmental: 500 mg/kg bw/d	Maternal: food intake and bw↓, abortion; Development: dead foetuses, malformations (external, visceral & skeletal)
Tasker et al., 1980*; TOX9552390; 98.7%; Monsanto	Dutch Belted rabbit, d 6-27 p.c., gavage	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 death at mid, 7 at high dose), soft stool, diarrhea; Development: none up to 175 mg/kg bw/d (high dose group excluded due to low number of foetuses and litters)

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

** study with serious deficiencies in conduct and reporting

In addition, the DS is aware of a single study with dietary administration of glyphosate (purity 96.8%, source most likely Alkaloida) to pregnant NZW rabbits. In this poorly reported study (Anonym, author perhaps Antal, 1981, TOX9650160), the test material was fed from gestation day 6 through 19 at three different dietary concentrations corresponding to daily intakes of 10.5, 50.7 or 255.3 mg/kg bw. Maternal toxicity was not observed. Likewise, there were no malformations noted and foetal weight was not affected. However, there was an increase in foetal losses at the two upper dose levels even though there was no the clear dose response (6.06 or 7.03% as compared to 0.93 or 0.79% in the control or low dose groups, respectively) that one would expect if the effect was really treatment-related. From the brief description, it appears that these findings were mostly post-implantation losses and, thus, would be somehow in line with what was observed in guideline-compliant gavage studies.

No published developmental studies in rabbits are available.

Excessive maternal toxicity became apparent mainly by a number of unscheduled, treatment-related deaths in 5 out of 7 studies in dose range from 100 to 500 mg/kg bw/day. In two studies (Tasker et al., 1980, TOX9552390; Suresh et al., 1993, TOX9551106), nearly one half of top dose animals was affected resulting in the loss of these dose groups for evaluation of developmental and teratogenic effects in foetuses. Mortality among pregnant does has been used to justify the proposal for classification of glyphosate for STOT RE and was therefore discussed in the respective section (see Table 18). Maternal toxicity was further characterised by gastro-intestinal clinical signs and reductions in food consumption and body weight or body weight gain. Sometimes, abortions were noted of which it is not clear whether they were due to maternal or instead to foetotoxicity. In any case, it must be acknowledged that all developmental findings in foetuses occurred in a dose range that was clearly toxic to the does even though there were differences among the studies with regard to severity of maternally toxic effects.

In spite of evident maternal toxicity, no developmental effects were observed in the study by Hojo (1995, ASB2012-11498) up to the top dose level of 300 mg/kg bw/day and in the study by Tasker et al. (1980, TOX9552390) up to the mid dose of 175 mg/kg bw/day, i.e., the highest dose at which foetuses could be evaluated. The other five studies deserve more detailed description since, here, developmental effects have been observed.

- In the study by Coles and Doleman (1996, ASB2012-11499), an increase in post-implantation losses was observed at the two upper dose levels, i.e., in the presence of maternal toxicity. The numbers of affected does were 10/15 at the mid dose and 9/15 at the high dose level as compared to 4/14 in the control group and 4/18 at the low dose level. In contrast, there was no increase in morphological anomalies.
- The study by Moxon (1996, TOX2000-2002), in contrast, revealed different developmental effects. Reduced foetal body weight and retarded ossification were observed at 300 mg/kg bw/day, again in the presence of maternal toxicity. No evidence of teratogenicity was obtained.
- The study by Suresh et al. (1993, TOX9551106) was compromised by high maternal mortality. During treatment, 4 does of the mid and 5 females in the top dose group died. In addition, further three high dose females died after scheduled cessation of substance administration. In principle, the premature death of more than one half of the pregnant rabbits at the high dose level would have required immediate termination of this group. From the beginning of the experiment, there were less does in the treated groups than in the control (15 to 17 mated females vs. 26). Together with the animal losses and a case of complete litter resorption, this difference resulted in a very low number of litters and foetuses from the

highest dose group that were available for teratological examination at scheduled sacrifice. An overview of foetal findings is given in Table 49.

The percentage of foetuses with ‘dilated heart’ was significantly increased at all dose levels. The diagnosis ‘dilated heart’ was not defined in the study report and neither criteria for this diagnosis nor any measurements of the heart and its size were provided. Because of the low number of foetuses and litters, it is hardly possible to interpret any of the results obtained in the top dose group. If only the low and mid dose group are considered and compared to the controls, the absolute number of foetuses and litters with ‘dilated heart’ was quite small and did not show a difference between the two groups although the dose applied to mid dose females was by five times higher. Thus, there was no clear dose response even though just this would be expected if it was a treatment-related effect.

In the presence of severe maternal toxicity, there was also a slight increase in the percentage of foetuses with extra 13^{th} rib.

In summary, the study results do not allow meaningful assessment developmental effects for the highest dose level. If assessment is confined to the low and mid dose levels, there was no clear evidence of foetotoxicity or teratogenicity because the finding ‘dilated heart’ was not really substantiated in the study report and because of the lacking dose response.

Table 49: Foetal findings in the study by Suresh et al. (1993, TOX9551106)

Dose group (mg/kg bw/day)	0	20	100	500
Percentage of foetuses with 'dilated heart'	0.0	5.1*	5.2*	17.9*
No. affected/total number of foetuses examined	-	4/78	4/77	5/28
Litters affected/no. of litters	-	3/13	2/12	2/6
Foetuses with major visceral malformations	4/133	6/78	6/77	8/28
Percentage of foetuses with extra 13th rib	0.0	1.3	2.6	3.6*

* statistically significant, p≤0.05

- The study by Brooker et al. (1991, TOX9552391) was of particular relevance since evaluation of developmental effects was feasible also at the top dose level of 450 mg/kg bw/day since the number of foetuses and litters was sufficient. The maternal NOAEL is based on clinical signs and decreased food consumption at 150 and 450 mg/kg bw/day. At the high dose level, one dam died following occurrence of clinical signs and abortion. The developmental NOAEL was established because of a higher frequency of late embryonic death at the highest dose level that was significantly elevated over the control value and was just at the upper edge of the historical control range. Furthermore, total embryonic losses were increased in all treated groups. However, this data is difficult to interpret since a comparison with historical control data from the performing laboratory proved a remarkably low percentage of post-implantation loss in the control group (5.7 %) that was below the historical control range (6.5-17.5 %). In contrast, the percentages for the low and high dose groups (19.5 and 21 %) were above its upper edge, but the 15.3% in the mid dose group was well within and there was no clear dose response. In this study, there was also an increase in cardiac malformations, mainly interventricular septal defects, at 450 mg/kg bw/day. This finding was observed in four foetuses from 4 litters as compared to one foetus showing this defect in each the control, low and mid dose groups. It must be emphasised that these malformations are apparently different from what is presumably defined by Suresh et al. (1993, TOX9551106) as 'dilated heart'.

Maternal and litter parameters from this study as well as an overview on foetal anomalies are given in Table 50 and Table 51.

Table 50: Summary of the maternal and litter parameters (group mean values) in the study by Brooker et al. (1991, TOX9552391)

Parameter	Dose Group (mg/kg bw/day)				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 does with live young or litters at Day 29	18	12	15	13	--

Parameter	Dose Group (mg/kg bw/day)				Historical control range (mean value)
	0 (Control)	50	150	450	
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)
Post-implantation loss (%)	5.7	19.5*	15.3*	21.0**	6.5 – 17.5 (12.9)
Live young	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
[#] Fisher exact test follow-up by intergroup comparison with control was not statistically significant P > 0.05

Table 51: Summary of foetal parameters in the study by Brooker et al. (1991, TOX9552391)

Parameter	Dose Group (mg/kg bw/day)				Historical control range or x/y ◊ (mean)
	0(control)	50	150	450	
Number of does with live young 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
No. of malformed foetuses	3	3	5	6	51
%	1.9	5.8	4.3	5.9 (F)	0.7 – 5.9 (3.8)
Number of Affected Litters	3	3	3	5	43/188
%	16.67	25	20	38.5	22.9
Thoracic region malformations					--
No. of foetuses with interventricular septal defect	1	1	1	4	10/1511
%	0.6	1.0	0.9	4.2	0.66
Litter incidence	1	1	1	4	10/188
%	5.56	8.3	6.67	30.8	5.32
Foetuses with enlarged left, reduced right ventricles	0	0	0	2	2/1511
%	0.0	0.0	0.0	2.1	0.13

Parameter	Dose Group (mg/kg bw/day)				Historical control range or x/y ◊ (mean)
	0(control)	50	150	450	
Litter incidence	0	0	0	2	2/188
%	0	0	0	15.4	1.10
Foetuses with retro-oesophageal right subclavian artery	0	0	3	2	7/1511
%	0.0	0.0	2.7	2.1	0.46
Litter incidence	0	0	1	1	7/188
%	0	0	6.6	7.6	3.72
Foetuses with narrow/dilated aortic arch/pulmonary trunk/arterial trunk	1	1	1	3	8/1511
%	0.6	1.0	0.9	3.2	0.52
Litter incidence	1	1	1	3	8/188
%	5.56	8.3	6.67	23.1	4.25
Anomalies					--
Total number of foetuses examined[#]	160	101	107	89	--
No. of foetuses with gross/visceral anomalies	9	14	14	6	--
%	6.4	19.5	12.9	9.6 (K)	--
No. of foetuses with skeletal anomalies	21	13	14	11	--
%	11.7	17.7	12.5	10.1 (K)	--
No. of foetuses with reduced ossification	7	4	5	4	--
%	4.4	4.0	4.7	4.5	--
Mean foetal weight of foetuses with reduced ossification (g)	37.9	43.6	37.7	26.1	--

◊ number affected / total number examined
[#] Malformed foetuses are excluded
(F) Fisher's exact test applied, not statistically significant (P > 0.05)
(K) Kruskal-Wallis 'H' statistic, not significant (P > 0.05)
-- no data

- The study of Bhide and Patil (1989, TOX9551960) was seriously flawed by serious deficiencies. Thus, no individual data is given and it is not clear whether statistical analysis of data has been performed and, if so, which statistical tests had been applied. Uterine weights and the results of maternal necropsy have not been reported. It is surprising that no maternal deaths have occurred even though the mid and high dose levels of 250 or 500 mg/kg bw/day had proven clearly toxic in other studies. It seems that the total number of foetuses and litters with malformations was higher in the groups receiving the mid and high doses of glyphosate but it is not clear whether they were found in different foetuses or if some foetuses had multiple malformations. The rather high number of visceral malformations at the top dose level was mainly due to absent kidneys or lung lobes, i.e., findings that can hardly be attributed to test substance administration. However, ventricular septal defects as in the study by Brooker et al. (1991, TOX9552391) were also noted but only in 2 out of 78 foetuses in the high dose group as compared to a control incidence of 0/109.

From all these studies, when taken together, the overall conclusion may be drawn that in rabbits, in contrast to rats, some developmental effects and, in addition, post-implantation losses have been

observed which can be allocated to glyphosate administration to the does. However, these findings were confined to dose levels at which severe maternal toxicity was apparent.

4.10.2.2 Human information

The same general constraints on the use of epidemiological data as discussed with regard to carcinogenicity and reproductive toxicity above (such as the lack of reliable exposure data, the impact of co-formulants or parallel exposure to other chemicals) apply also to developmental toxicity and teratogenicity. So far, there is no convincing evidence that exposure to glyphosate formulations will increase the risk for an adverse developmental outcome in humans.

Two studies on residential proximity to agricultural pesticide applications in California by and examined whether early gestational exposure to pesticides was associated with an increased risk of hypospadia (Carmichael et al., 2013, ASB2014-9307) or neural tube defects and orofacial clefts (Yang et al., 2013, ASB2014-9644) in offspring. In both studies formulated glyphosate (mentioned as ”phosphonoglycine”) was included in the analyses and exposure was frequent but no positive correlation was found.

In a study from Ontario (Canada), Arbuckle et al. (2001, ASB2012-11545) reported a slight increase in the pre-conception glyphosate exposure odds ratio for spontaneous abortion of borderline significance (OR = 1.4). Due to strong limitations in this study, no firm conclusion is possible. Thus, 395 spontaneous abortions were reported out of 3936 pregnancies giving a rate of spontaneous aborting of 10% that is below the baseline rate in the general population of 12 to 25 %. Recall bias is reflected in the recall of spontaneous abortion over the previous 5 years (64 % of all spontaneous abortions reported) being much higher than the recall of those greater than 10 years prior to the survey (34 % of all spontaneous abortions reported).

There are some reports from South America claiming an increasing frequency of birth defects in rural areas where the population is heavily exposed to agrochemicals (e.g., Campana et al., 2010, ASB2013-10559). Lopez et al. (2012, ASB2013-10534) also reported an increase in malformations but also in cancer incidence from certain regions but these increases were more general without clear-cut evidence of a distinct anomaly or a certain cancer type. The general weaknesses of such data collected in so-called ”ecological” (“correlational”) studies are the unknown exposure level and the impossibility to attribute a certain outcome to exposure to a single substance (Paumgartten et al., 2012, ASB2013-10538). There is no evidence so far that the reported increases might be related to glyphosate. Thus, Benitez-Leite et al. (2009, ASB2012-11563) reported the incidence of anomalies in newborn babies in a hospital in Paraguay but from this data it cannot be concluded if there was in fact an increase. Many of the reported anomalies were variations rather than malformations and, according to inquiries by the RMS, a similar incidence might be expected in an average German birth clinic. Furthermore, a single “hospital-based” analysis is not sufficient to prove changes in the prevalence of malformations in a region. The authors themselves reported a (not specified) “high” exposure of the parents to agrochemicals and pesticides in general but glyphosate or glyphosate-containing herbicides were not explicitly mentioned. In everyday life, people in these rural areas were exposed to a great number of agrochemicals that, taken together, might result in a higher risk for adverse outcomes such as malformations or cancer, in particular if exposure is high and appropriate safety measures are not taken. However, this assumption is of not much use neither for risk assessment for a single substance nor for its classification and labelling. Even if the claimed increases could be substantiated in future, it is unlikely that they were due to glyphosate, taking into account the extensive toxicological database and the long history of its worldwide safe use.

The absence of reproductive and developmental effects in humans is not surprising since human *in utero* exposures would be very limited. On one hand, the perfusion rate of glyphosate across the

placenta is low (Mose et al., 2008, ASB2012-11914). On the other hand, systemic intake of glyphosate in the general population is low. McQueen et al. (2012, ASB2012-11898) calculated a very low dietary exposures of pregnant women in Australia ranging from 0.005 to 2 % of the ADI of 0.3 mg/kg bw for glyphosate as established by the Australian authorities. In combination, both facts will contribute to a nearly negligible *in utero* exposure.

4.10.3 Other relevant information

There are a large amount of *in vitro* and a few *in vivo* studies on different aspects of reproductive and developmental toxicity of glyphosate and its formulations for which the reader is referred to the attached Vol. 3 of the RAR. For purposes of classification and labelling, this often contradictory information is not that useful since there is a sufficient and adequate database of higher tier animal studies that have been performed in compliance to current guidelines employing very high doses.

However, it should be highlighted that glyphosate was found to be devoid of a potential for endocrine disruption in recent testing on request of U.S. EPA. Glyphosate was included into the U.S. EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds that were foreseen to Tier 1 Screening. The compounds were selected on their potential for exposure rather than suspected interference with the endocrine system and tested for their potential to interact with the oestrogen, androgen and thyroid endocrine pathways. Levine et al. (2012, ASB2014-9609) published a short summary of the results. According to this, very brief information, glyphosate was tested in Tier 1 assays for (anti-)estrogenic and (anti-)androgenic properties and an impact on steroidogenesis *in vitro*. *In vivo* testing comprised the uterotrophic, Hershberger and male and female pubertal assays. These tests were performed at different laboratories. Bailey et al. (2013, ASB2013-3464) summarized the first results of the male and female pubertal assays in which glyphosate did not exhibit evidence of endocrine disruption.

Based on this new data and on the outcome of the reproductive and developmental studies in animals, the DS does not consider glyphosate to be a substance with endocrine disrupting properties.

In the past, two reports on a teratogenic potential of glyphosate gained notable public attention and are discussed here briefly.

Paganelli et al. (2010, ASB2012-11986) exposed embryos of the clawed frog Xaenopus laevis to a glyphosate formulation via the water or via injection of the test substance directly into frog embryos. In another experiment and, chicken embryos were exposed directly to a glyphosate formulation through a hole cut in the egg shell. The authors claimed to have found evidence of teratogenicity, in particular of neural crest lesions that might progress to craniofacial malformations. A mechanism similar to that of excess retinoic acid was suspected. However, the relevance of these findings must be questioned because of highly artificial routes of exposure as well as the application of excessive doses. Craniofacial malformations were not noted in developmental studies in rats or rabbits. Decisions on classification and labelling are mainly based on effects in adequate studies in mammals and not on mechanistic considerations.

Krüger et al. (2014, ASB2014-8935) reported glyphosate residues in different organs/tissues (brain, gut wall, heart, kidneys, liver, lungs, and muscle tissue) from a total of 38 malformed one-day old piglets (breed not specified) which had been brought in by a Danish farmer. Various, very different malformations were seen, including craniofacial but also visceral and leg anomalies. For determination of glyphosate, apparently the same ELISA as for urine measurements (Abraxis, USA) was used after mincing and diluting tissue samples from the various organs. Its previous validation for the new matrix was not reported and no LOD or LOQ were mentioned. Mean glyphosate concentrations between 2.1 ppm (liver) and 12.9 ppm (heart) were found. For most organs, the standard deviation was extremely large and individual values in single animals ranged from 0 (liver)

and 0.1 ppm (kidney) to occasional findings as high as 80 ppm in lung and heart. The authors speculated if there was a correlation between the malformations and intake of glyphosate residues to which the piglets might have become exposed via the placenta. The farmer claimed that the rate of malformed piglets had increased from 1:1432 when the sows had been fed a diet containing 0.25 ppm glyphosate to 1:260 when the sows received a diet with a glyphosate content of 0.87-1.13 ppm during the first 40 days of pregnancy. This publication cannot be considered as describing a reliable scientific study. Apart from the analytical uncertainties, the main weakness of the study is that only malformed piglets had been investigated for glyphosate concentrations in their organs. Thus, there was no control group to prove the hypothesis of a potential correlation.

Such a correlation is unlikely because of the following considerations:

- In a multitude of developmental studies and multi-generation studies in rats, no evidence of teratogenicity was obtained. Even in rabbits which proved more vulnerable, developmental effects were confined to exaggerated dose levels which also caused clear maternal toxicity. It is very unlikely that pigs, receiving much lower amounts of glyphosate by ingestion of residues in the diet, should be that much more sensitive and, if so, it is hardly conceivable that such effects would not have become apparent earlier and also in other countries and on other farms.
- Many different malformations were reported. However, most chemical teratogens produce a specific teratogenic effect or a certain pattern of findings. Moreover, teratogenic effects usually follow a dose response relationship. In this case, the glyphosate concentrations in the organs and tissues were so variable that such a dose response relationship may be excluded.
- Malformations in piglets are quiet frequent and often have a genetic background. Infectious diseases may also play a role. There is no indication in the paper that an alternative diagnosis had been considered.

4.10.4 Summary and discussion of reproductive toxicity

There was a very large database submitted by different applicants and from published scientific literature to evaluate reproductive and developmental toxicity of glyphosate. At least six valid multi-generation studies in rats, six developmental toxicity studies in rats and seven developmental toxicity studies in rabbits have been evaluated. All available data were considered together using a weight of evidence approach with consideration of the biological significance, maternal toxicity and the consistency of the reproductive and developmental findings.

In the rat, there was no evidence of specific reproductive toxicity or of a teratogenic potential since effects, if observed at all, were very weak and confined to very high dose levels causing already some parental or maternal toxicity.

In the developmental studies in rabbits some adverse developmental effects have occurred only in the presence of maternal toxic effects for which a comparison with criteria is needed (see below).

No convincing evidence of reproductive or developmental effects of glyphosate may be derived from epidemiological studies or from *in vitro* or *in vivo* studies on different aspects of reproduction.

4.10.5 Comparison with criteria

4.10.5.1 Effects on fertility

The following criteria for classification for adverse effects on sexual function and fertility are given in CLP regulation:

CLP criteria
Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies — clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or — the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant — 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 (deficiencies in the study). — the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

Reproductive studies in rats have clearly shown that these criteria were not met.

4.10.5.2 Developmental toxicity

The following criteria for classification for adverse effects on development are given in CLP regulation:

CLP criteria
Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies — clear evidence of an adverse effect on development in the absence of other toxic effects, or — the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant — some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and — the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). — the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

General remark: For the majority of chemical substances evaluated under the CLP-Regulation, normally one study addressing developmental toxicity in the rats and rabbits, respectively is required and therefore available for classification and labelling purposes. In contrast, for glyphosate, a large quantity of animal data regarding developmental toxicity is available, and six developmental toxicity studies in rats and seven developmental toxicity studies in rabbits have been evaluated. Therefore, all available data from all studies were considered together using a weight of evidence approach. Basing any conclusion only on the statistical significance of an increased incidence of a finding identified in a single study without consideration of the biological significance, the influence of maternal toxicity and the consistency of the developmental findings should be avoided.

Category 1A does not apply since there are no reliable human data and epidemiological studies that would provide convincing evidence of teratogenicity to humans.

Whereas the results of the studies in rats were not of concern, the cardiac malformations (i.e., interventricular septal defects) in rabbit foetuses have provoked a lot of controversial discussions (e.g., Antoniou et al., ASB2012-15927; Kimmel et al., 2013, ASB2013-3462). They are discussed in the following in greater detail and compared with the criteria for categories 1B and 2.

These findings were observed in few foetuses at various dose levels including the control. An increase was confined to the very high dose levels of 450 mg/kg bw/day (Brooker et al., 1991, TOX9552391) and 500 mg/kg bw/day (Bhide and Patil, 1989, TOX9551960), with the latter being a study of questionable reliability. The effect dose of 450 mg/kg bw/day was clearly in a dose range that is toxic to pregnant rabbits. In the Guideline-compliant study of Brooker et al. (1991, TOX9552391), a higher frequency of interventricular septal defects was indeed associated with some maternal toxicity including one death following abortion, gastrointestinal signs and slightly lower food consumption and body weight gain. When all the rabbit studies are taken together, first deaths were observed at a dose level of 100 mg/kg bw/day or 175 mg/kg bw/day and excessive toxicity resulting in the loss of nearly one half of the does was observed from 350 mg/kg bw/day onwards (Suresh et al., 1993, TOX9551106; Tasker et al., 1980, TOX9552390). Mortality was also seen at high dose levels in the studies by Coleman and Doles (1996, ASB2012-11499), Hojo (1995, ASB2012-11498) and Brooker et al. (1991, TOX9552391) even though the number of affected does was lower. Gastrointestinal signs, abortion and post-implantation losses also suggest severe maternal toxicity. As shown above, it is proposed to classify glyphosate as STOT RE for the maternal deaths in pregnant rabbits.

Despite administration of high doses, interventricular septal defects were not observed in two further studies in NZW rabbits from the mid-90s (Coleman and Doles, 1996, ASB2012-11499; Moxon, 1996, TOX2000-2002). Moreover, such findings were not reported in another rabbit strain (Hojo, 1995, ASB2012-11498). In fact, the top dose levels in these studies were lower (300 or 400 mg/kg bw/day) but, on the other hand, it would have been hardly possible to increase the maximum doses without causing excessive maternal toxicity.

The study by Suresh et al. (1993, TOX9551106) cannot not be taken as supportive evidence for cardiac malformations because the heart findings there (‘dilated heart’) were of a completely different nature. Dose response for this ‘dilatation’ was questionable, description of the findings was poor and a similar effect was not reported in other studies. Thus, it seems reasonable to disregard this equivocal finding with regard to classification and labelling.

Category 1B is not applicable because the higher incidence of interventricular septal defects at 450 mg/kg bw/day was associated with marked maternal toxicity in the same study (Brooker et al., 1991, TOX9552391) and even more pronounced maternal effects at lower doses in other rabbit studies. Thus, adverse developmental effects have occurred only in the presence of other toxic effects. It may be concluded that an increased risk for foetal heart effects in rabbit foetuses was confined to levels of exposure that also caused severe maternal toxicity. Therefore, and taking into consideration the rather low foetal incidence of interventricular septal defects at 450 mg/kg bw/day and their complete absence at 400 mg/kg bw/day in another study in the same strain (Coleman and Doles, 1996, ASB2012-11499), it may be assumed that this finding is a non-specific secondary consequence of marked maternal toxicity. Accordingly, category 2 would be also not appropriate.

4.10.6 Conclusions on classification and labelling

No classification and labelling of glyphosate for reproductive or developmental effects is proposed.

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 *et al*., 2007; 95.7%	Sprague-Dawley, diet	0, 1500, 5000, 15000 ppm (corresponding to approximately 0, 105, 351	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

		and 1053 mg/kg bw/d)			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; 97.6%	Wistar-derived AlpK, diet	0, 1000, 3000, 10000 ppm (corresponding to approximately 0, 100, 293 and 985 mg/kg bw/d)	Parental, offspring: 3000 ppm (293 mg/kg bw/d); Reproductive: 10000 ppm (985 mg/kg bw/d)	Parental, offspring: 10000 ppm (985 mg/kg bw/d); Reproductive: not established	Parental, offspring: 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 *et al*., 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-depended 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 (ADD/ADHD) in children. However, a statistically significant positive association for these findings is 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 *et al*., 1991, 98.6% (acceptable or at least supplementary in RAR)	CD, gavage, GD 6-15	0, 300, 1000, 3500 mg/kg bw/d	Maternal & developmental: 300 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Maternal: two 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 sternebrae (%)	13.7	28.5	17.6	33.8**
Foetuses showing skeletal variation (%)[1]	11.7	22.6	28.4	35.7**

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

[1]Historical 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 rabbits[1] (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 *et al*., 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 viceral malformations in all dose groups (dilated heart)
Brooker *et al*., 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 bw/d dose groups	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, 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 *et al*., 1980*; 98.7%. Adhere to GLP (study supplementary in RAR)	Dutch Belted rabbit, GD 6-27, gavage. 16, 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 litteres 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

[1]Detailed 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 I: 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 ± 5.6g, 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 ± 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 slighthly 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)**
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 foetuses in 15 litters as compared to 50 foetuses in 15 litters in the controls) or 27 pre-sacral vertebrae (37 foetuses 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 foetuses 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 foetuses 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 exmined	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 fooetuses/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 ◊ (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					**--**
Total number of foetuses examined[#]	160	101	107	89	--
Number of foetuses with gross/visceral anomalies (%)	9 (6.4)	14 (19.5)	14 (12.9)	6 (9.6 (K))	--
Number of foetuses with skeletal anomalies (%)	21 (11.7)	13 (17.7)	14 (12.5)	11 (10.1 (K))	--
Number of foetuses with reduced ossification (%)	7 (4.4)	4 (4.0)	5 (4.7)	4(4.5)	--
Mean foetal weight of foetuses with reduced ossification (g)	37.9	43.6	37.7	26.1	--

◊ Number affected / total number examined

[#] Malformed foetuses 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 foetuses

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 foetuses and litters with malformations were higher at 250 and 500 mg/kg bw/d relative to controls (3 foetuses (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) foetuses (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 form the reporting of the study whether the different malformations were found in different foetuses or if some foetuses 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 foetuses where 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 foetuses in the low-, mid- and high-dose group, respectively) or splitting (0, 0, 3 and 1 foetuses in the low-, mid- and high-dose group, respectively) of the parietal bones. In the low- and high-dose groups, 1 foetus and 2 foetuses 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 foetuses are affected, and for most of the skeletal malformations 1-2 foetuses/litter were affected. Visceral malformations were reported in one foetus 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 foetuses from the same litter had fusion of the right pulmonary lobe and one of the foetuses also had undescended testis. One foetus 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 foetuses (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 foetuses 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 foetuses 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.

<u>Human information</u>

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 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.

Supplemental information - In depth analyses by RAC

Table A below summarise data on group mean foetal weight, post-implantation loss and viable litter size at C-section from the seven rabbit developmental toxicity studies that are available for glyphosphate.

Table A. Summary of litter data from the available rabbit developmental toxicity studies (data is taken from the original study reports)

Study (dose)	Parameter	Dose level			
		Control	Low dose	Intermediate dose	High dose
Coles and Doleman, 1996 (0, 50, 200 & 400 mg/kg bw/d) *p<0.05	Number of dams with viable foetuses at scheduled C-section	14	18	15	15
	Number of dams with total litter loss at scheduled C-section	0	0	0	0
	Mean number of implantations/dam	9.5±2.5	9.1±2.3	8.9±2.5	10.3±2.3
	Mean number of early embryonic/foetal deaths/dam	0.21±0.43	0.22±0.55	0.87±1.06	0.47±0.92
	Mean number of late embryonic/foetal deaths/dam	0.14±0.53	0.11±0.32	0.13±0.35	0.93±2.28
	Mean number of total embryonic/foetal deaths/dam	0.36±0.63	0.33±0.77	1.00*±1.00	1.40±2.35
	Mean percentage of post-implantation loss/dam	3.7±6.5	3.6±8.5	11.5±11.4*	12.1±18.6
	Mean number of live foetuses/litter	9.1±2.5	8.7±2.4	7.9±2.5	8.9±2.6
	Mean foetal weight/litter (g)	41.5±5.5	39.4±5.6	41.7±4.5	38.2±5.2
Moxon, 1996 (0, 100, 175 & 300 mg/kg bw/d) *p<0.05	Number of dams with viable foetuses at scheduled C-section	17	18	17	17
	Number of dams with total litter loss at scheduled C-section	0	0	0	0
	Mean number of implantations/dam	9.65±2.06	9.00±1.78	9.12±2.5	9.82±1.88

	Mean percentage of early intrauterine death/dam	6.2±9.7	7.5±17.0	8.1±8.1	11±16
	Mean percentage of late intrauterine deaths/dam	5.5±10.4	1.9±4.5	4.0±4.9	2.5±8.3
	Mean percentage of post-implantation loss/dam	11.7±12.0	9.5±16.7	12.1±9.7	13.6±16.6
	Mean number of live foetuses/litter	8.41±1.80	8.17±2.20	7.94±2.19	8.47±2.32
	Mean foetal weight/litter	44.4±4.3	43.3±3.9	43.2±5.7	40.7±7.8*
Brooker *et al.*, 1991 (0, 50, 150 & 450 mg/kg bw/d) *p<0.05 **p<0.01 No information available on standard deviations (SD) for the calculations.	Number of dams with viable foetuses at scheduled C-section	18	12	15	13
	Number of dams with total litter loss at scheduled C-section	0	0	0	0
	Mean number of implantations/dam	9.7	10.5	9.0	9.2
	Mean number of early embryonic deaths/dam	0.4	0.9	0.9	0.5
	Mean number of late embryonic deaths/dam	0.2	0.9	0.5	1.3**
	Mean number of total embryonic death/dam	0.6	1.8*	1.5*	1.8**
	Mean percentage of post-implantation loss/litter	5.7	19.5*	15.3*	21.0**
	Mean number of live foetuses	9.1	8.7	7.5	7.3
	Mean foetal weight	43.9	43.3	44.0	44.5
Suresh *et al.*, 1993 (0, 20, 100 & 500 mg/kg bw/d) # no info on SD * p≤0.05.	Number of dams with viable foetuses at scheduled C-section	20	13	12	5
	Number of dams with total litter loss at scheduled C-section (data included in calculations)	0	0	0	1
	Mean number of implantations/dam	8±2.0	8±1.5	9±1.8	6±2.4
	Total number of embryonic resorptions/group (%)	10 (7)	11 (11)	11 (11)	9 (24)
	Total number of foetal resorptions/group (%)	8 (5)	7 (7)	13 (13)	1 (3)
	Total number of post-implantation loss/group (%)	18 (12)	18 (18)	24 (24)	10 (26)

	Mean number of viable foetuses/litter[#]	7	6	7	6
	Mean foetal body weight	32±5.3	35±3.7*	35±2.4*	33±4.9
Bidhe and Patil, 1989[1] 0, 125, 250, 500 mg/kg bw/d)	Number in the study	15	15	15	15
	Number aborted	0	0	0	2
	Number non-pregnant at termination[2]	2	1	1	3
	Number pregnant at termination	13	14	14	12
	Number with no live fetuses[3]	0	0	0	2
	Number of litters examined	13	14	14	12
	Mean number of implantations/dam[4]	9.0±1.2	9.3±1.3	9.4±1.12	8.5±1.05
	Mean number of early resorption/dam[4]	1.7±3.22	1.1±2.53	1.0±2.56	1.9±2.43
	Mean number of non-viable implants/dam[4]	0.07±0.26	0.13±0.35	0.27±0.59	1.4±2.2
	Mean number of viable implants/dam[4]	7.3±3.1	8.0±2.59	8.0±2.48	5.2±3.03
	Mean foetal body weight[4]	40.6±16.6	47.1±0.95	47.5±1.38	48.7± 1.87
Hojo, 1995 (0,10,100, 300 mg/kg bw/d)	Number of dams with total litter loss at scheduled C-section (not included in calculations)	0	1	2	1
	Number of dams with viable litters at scheduled C-section	18	15	16	14
	Mean number of implantations/dam	8.5±2.8	9.8±2.9	10.4±2.9	8.6±3.3
	Mean number of live foetuses/dam	7.8±2.4	8.7±3.2	9.4±2.7	8.0±3.2
	Percentage fetal resorptions and deaths	7.1	13.8	8.7	6.5
	Mean foetal body weight (M)	35.8±8.1	37.3±5.4	36.7±3.3	36.2±5.4
	Mean foetal weight (F)	35.7±6.7	36.1±5.1	36.0±3.9	34.9±4.4
Tasker *et al*., 1980 (0, 75, 175, 350 mg/kg bw/d) *p<0.05	Number of dams with viable litters at scheduled C-section	12	15	11	6
	Number of dams with total litter loss at scheduled C-section	0	0	0	0

	Mean number of implantations/dam	5.9±2.39	8.0±1.81	6.1±2.84	7.2±2.93
	Mean number of post-implantation loss/dam	0.7±0.89	0.4±0.63	0.2±0.4	0.8±1.33
	Mean number of early resorptions/dam	0.4 ± 0.9	0.3±0.59	0.1±0.3	0.5±0.84
	Mean number of late resorptions/dam	0.3±0.45	0.1±0.35	0.1±0.3	0.3±0.52
	Mean number of viable foetuses/dam	5.3±2.73	7.6*±1.84	5.9±2.77	6.3±2.25
	Foetal body weight	33.4±7.27	30.9±4.43	29.9±7.21	29.3±4.82

[1] Study with serious deficiencies in conduct and reporting, thus the data is presented exactly as reported in the summary table I of the study report.
[2] Normally the term "non-pregnant" is used to define animals that have no implantations at C-section. As revealed from the individual litter data in the study report, all animals in the study had implantations and it appears that the "non-pregnant animals" in fact were animals that had total litter loss.
[3] This data is not in line with the data presented in the individual litter data.
[4] Data from "non-pregnant" as well as female rabbits that aborted during the study have been included in the calculations.

Table B

Summary of malformations in the rabbit developmental toxicity studies (data taken from study reports).

Study (dose)	**Parameter**	**Dose level**			
		Control	**Low dose**	**Intermediate dose**	**High dose**
Tasker *et al*., 1980 (0, 75, 175, 350 mg/kg bw/d)	Number of fetuses (litters) examined	63(12)	114(15)	65(11)	38(6)
	Total number of fetuses (litters) with malformations	0	3(3)	2(2)	2(1)
	- External/Visceral - Skeletal - Cardiovascular	0 0 0	0 3(3) 0	0 2(2) 0	2(1) 0 0
Suresh *et al*., 1993 (0, 20, 100 & 500 mg/kg bw/d) #significantly different from control by Contingency testing	Number of fetuses (litters) examined	133(20)	79(13)	77(12)	28(5)
	Total number of fetuses (litters) with malformations	Not reported	Not reported	Not reported	Not reported
	- External - Visceral - Skeletal - Cardiovascular	2(2) 4(3) 11(4) 2(2)	2(1) 6(3) 5(3) 4(3)	1(1) 6(4) 0# 6(4)	0 8#(2) 1(1) 6(2)
Moxon, 1996	Number of fetuses (litters) examined	143(17)	147(18)	135(17)	144(17)

(0, 100, 175 & 300 mg/kg bw/d)	Total number of fetuses (litters) with malformations	3(2)	1(1)	0	2(2)
	- External/Visceral	2(2)	1(1)	0	2(2)
	- Skeletal	3(2)	0	0	1(1)
	- Cardiovascular	1(1)	1(1)	0	1(1)
Hojo, 1995 0,10,100, 300 mg/kg bw/d) *p≤0.05	Number of fetuses (litters) examined	140(18)	130(15)	150(16)	112(14)
	Total number of fetuses (litters) with malformations	1(1)	Not reported (3)	Not reported(3)	5(5*)
	- External	0	0	2(1)	0
	- Visceral	0	1(1)	3(2)	0
	- Skeletal	1(1)	4(3)	6(2)	5(5*)
	- Cardiovascular	0	0	1	0
Coles and Doleman, 1996 (0, 50, 200 & 400 mg/kg bw/d)	Number of fetuses (litters) examined	128(14)	157(18)	119(15)	134(15)
	Total number of fetuses (litters) with malformations	2(2)	3(2)	2(2)	1(1)
	- External/Visceral	1(1)	2(1)	2(2)	1
	- Skeletal	1(1)	1(1)	1(1)	0
	- Cardiovascular	0	0	1(1)	0
Brooker *et al*., 1991. (0, 50, 150 & 450 mg/kg bw/d)	No of fetuses (litters) examined	163(18)	104(12)	112(15)	95(13)
	Total no. fetuses (litters) with malformations[1]	3(3)	3(3)	5(3)	6(5)
	- Cardiovascular	1(1)	1(1)	4(2)	5(4)
Bidhe and Patil[2], 1989. (0, 125, 250, 500 mg/kg bw/d)*	Number of fetuses (litters) examined	109(13)	113(14)	120(14)	78(12[2])
	Total no. fetuses (litters) with malformations	3(3)	6(6)	10(10)	20(14[2])
	- External	1(1)	2(2)	3(3)	3(3)[2]
	- Visceral	1(1)	4(4)	5(5)	12(9)[2]
	- Skeletal	1(1)	0	2(2)	5(2)[2]
	- Cardiovascular	0	1(1)	1(1)	2(2)[2]

[1]The study report only presented summary information regarding number of foetuses (litters) with malformations.

[2] Study with serious deficiencies in conduct and reporting The reporting of the data is unclear. The total number of litters in the high dose with malformations is reported to be 14. However, the number of animal 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 foetuses where examined and that data from these 2 litters were included in the analysis. Consequently, it is unclear to what extent the data for the high dose group represents finding in aborted foetuses**.**

RAC evaluation of aspiration toxicity
This hazard class was not evaluated.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 52: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolyses determination of glyphosate at different pH values US EPA 540/9-85-013, Series 161-1	Glyphosate, purity 96.6% In range of pH 5-9 stable, no hydrolysis products were detected	Accepted during EU review (2001)	Burgener (1990)
Photodegradation study of glyphosate in water at pH 5,7 and 9 US EPA 540/9-82-021, Series 161-2	Glyphosate, purity 96.6% DT_{50} = 33 d (pH 5) DT_{50} = 69 d (pH 7) DT_{50} = 77 d (pH 9)	Accepted during EU review (2001)	Van Dijk (1992)
Biodegradation OECD 302 B, 1981	Glyphosate, purity 96.6% 0 % after 28 days	Accepted during EU review (2001)	Wüthrich (1990)
Biodegradation OECD 302 B, 1981	Glyphosate, purity 96.6% 2 % after 28 days	Accepted during EU review (2001)	Carrick (1991)
Biodegradation OECD 301 F	< 60 % after 28 days	Study report not available	Feil (2009)

5.1.1 Stability

The hydrolysis study with glyphosate (Burgener (1990, BVL no 2442046) was assessed as acceptable during the EU review of glyphosate (2001). The results are summarised in the monograph of glyphosate:

Solutions of ^{14}C-1-methane glyphosate (purity 96.6 %) in water at pH 5, 7 and 9 were reacted in the dark under sterile conditions at 25 °C for 30 days. After an incubation time of 30 days, no hydrolysis products were detected in the test solution and no significant amount of volatile products were observed in the absorption traps (<0.1 %). In the pH range 5 to 9 tested glyphosate is stable towards hydrolysis.

The photochemical degradation of glyphosate was investigated during the 2001 EU approval of glyphosate. The results of the acceptable study with glyphosate (van Dijk, 1992, BVL no 2252558) are summarized in the Monograph of glyphosate:

The rate of photolysis of ^{14}C-1-methane glyphosate was determined in distilled and sterile water solutions after 0,1,4,7 and 16 days at pH of 5.1, 7.3 and 9.2 at 25 °C in a suntest irradiation apparatus simulating natural sunlight. At every pH, the parent compound was not significantly degraded in the dark, i.e. the amount of parent compound from day 0 to day 15 did not decrease more than 3.5 %. The half-lives of glyphosate are a function of solution pH: at pH 5 (DT_{50} of 33 days), at pH 7 (DT_{50} of 69 days) and at pH 9 (DT_{50} of 77 days).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

In the 2001 EU evaluation of glyphosate, several studies assessing glyphosate's ready biodegradability have been reviewed. Two out of these reviewed studies were conducted according to the OECD guideline 302 for test on inherent biodegradability (Wüthrich, 1990, BVL no 1934369; Carrick, 1991, BVL no 2325628). An additional study according to OECD guideline 301 F (Mamometric Respirometry Test) was prepared by a Glyphosate Task Force (GTF) member (Feil, 2009).

In all studies, glyphosate did not show mineralisation of more than 60 % within 28 days. Therefore, the active substance is classified as not ready biodegradable. Table 47 summarizes all the available compliant studies mentioned above.

The study of Feil (2009) was not presented to the RMS and therefore could not be checked. However, the results presented in the dossier of the notifier are in line with the available studies and therefore are plausible.

Table 53: Overview of the glyphosate biodegradability studies

Reference		Guideline	Inocolum	Conc. (g dry material/L)	Test Conc. (mg/L)	Fraction of CO_2 produced from parent	
						Functional control	Glyphos-ate
Studies from the 2001 Evaluation	Wüthrich, 1990, BVL no 1934369	OECD 302 B, 1981	1. Sludge from domestic WTP (CH) 2. Sludge from WTP of Cheminova (DK)	0.2	620	88 % and 89 % within 7 days	0 % after 28 days for both systems
	Carrick, 1991, BVL no 2325628	OECD 302 B, 1981	Activated sludge from Kendal WTP	0.2	250	100 % within 2 days	2 % after 28 days
New study	Feil, 2009	OECD 301 F	Activated sludge from Darmstadt (Germany) WTP	1.5	103	98 % after 28 days	< 60 % after 28 days

Conc. = concentration; WTP = waste water treatment plant

5.1.2.3 Simulation tests

5.1.3 Summary and discussion of degradation

The study on ready biodegradability according to OECD 301 F (Manometric Respirometry Test) shows that glyphosat is not readily degradable (< 60 % degradation at 28 days).

The study on inherently biodegradability according to OECD 302 B (Modified Zahn Wellens Test) shows that glyphosat is not rapidly degradable (0-2 % degradation at 28 days).

Glyphosat is hydrolytically stable under acidic and neutral conditions. Aquatic photolysis is not considered as an important transformation route for glyphosate in the environment with DT_{50} of 33 – 77 days.

The results of the tests on the biodegradation of glyphosat show that glyphosate is not rapidly degradable (a degradation > 70 % within 28 days) for purposes of classification and labelling.

5.2 Environmental distribution

Not relevant for this dossier.

5.3 Aquatic Bioaccumulation

Table 54: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water EEC A 8 shake flask	log $P_{o/w}$ < - 1.3 (measured)	accumulation potential in aquatic non-target organisms is hence considered to be low	Wollerton and Husband (1997)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Glyphosate acid has a log P_{OW} value of < -1.3. Therefore, based on the low log P_{ow}-values the potential for bioconcentration is considered negligible. The octanol/water partition coefficient of glyphosate acid, expressed as log P_{ow}, is < -1.3. Values less than 3 indicate a low potential for bioaccumulation, therefore no further assessment is necessary.

5.3.1.2 Measured bioaccumulation data

No data available.

5.4 Aquatic toxicity

Table 55: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Acute toxicity of Glyphosate acid to Bluegill Sunfish (*Lepomis macrochirus*) OECD 203/FIFRA 72-1 Static exposure	96 hour LC_{50} = 47 mg/L (nominal) with a 95 % confidence interval of 35 to 66 mg/L	--	Kent, S.J., Caunter, J.E., Morris, D.S., Johnson,P.A. (1995)
Chronic Toxicity of Glyphosate acid to zebra fish larvae (*Brachydanio rerio*) OECD 212 semi-static exposure	NOEC (168 h) = 1.0 mg/L (nominal)	recalculated value key study	Dias Correa Tavares, C.M. (2000)
Acute toxicity of Glyphosate acid to *Daphnia magna* OECD 202 Static exposure	LC_{50} (48 h) = 84 mg/L (nominal) with a 95 % confidence interval of 73.3 to 101 mg/L	--	Wüthrich, V. (1990)
Glyphosate acid: Chronic toxicity to *Daphnia magna* OECD 202, part II semi-static exposure	NOEC (21 d) = 12.5 mg/L (nominal) for reproduction	--	Magor, S.E., Shillabeer, N. (1999)
Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum* OECD 201 Static exposure	E_rC_{50} (72 h) = 18 mg/L (nominal) with a 95 % confidence interval of 10 to 42 mg/L NOE_rC (72 h) = 1.82 mg/L (nominal)	--	Smyth, D.V., Kent, S.J., Morris, D.S., Shearing, J.M., Shillabeer, N. (1996)
Glyphosate acid: Toxicity to blue-green alga *Anabaena flos-aquae* OECD 201 Static exposure	E_rC_{50} (72 h) = 22 mg/L (nominal) with a 95 % confidence interval of 8.8 to >96 mg/L NOE_rC (72 h) = 12 mg/L (nominal)	--	Smyth, D.V., Shillabeer, N., Morris, D.S., Wallace, S.J. (1996)
Glyphosate acid: Toxicity to duckweed (*Lemna gibba*) EPA FIFRA Guideline 123-2 semi-static exposure	EC_{50} (14 d) = 12 mg/L (nominal) with a 95 % confidence interval of 11 to 14 mg/L for inhibition of frond number NOEC (14 d) = 3 mg/L (nominal) for inhibition of frond number	--	Smyth, D.V., Kent, S.J., Morris, D.S., Cornish, S.K., Shillabeer, N. (1996)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1

Author:	Kent, S.J.,Caunter, J.E.,Morris, D.S., Johnson,P.A.
Title:	Glyphosate acid: Acute toxicity to Bluegill Sunfish (*Lepomis macrochirus*)
Date:	21.12.1995
Doc ID:	2310926 /BL5553/B
Guidelines:	OECD 203/FIFRA Guideline 72-1
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 % a.s.
Control:	Filtered and dechlorinated tap water
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	Juvenile
Size:	30 mm (mean)
Body weight:	0.54 g (mean)
Loading:	10 test individuals for 20 L test solution
Source:	Aquatic Research Organisms, Hampton, New Hampshire, USA
Diet/Food:	no feeding for 48 hours prior to test and during the total test period
Acclimation period:	19 days at 22 °C prior to the test initiation
Temperature:	22 ± 1 °C
Photoperiod:	16 hours with 20 min transition period
pH:	Control (start – 96 h): 7.3–6.8 10 mg/L (start – 96 h): 5.9 – 6.4 18 mg/L (start – 96 h): 5.2 – 5.8 32 mg/L (start – 96 h): 4.6 – 4.8 56 mg/L(start – 96 h): 3.8 – 3.9 100 mg/L (start – 24 h): 3.4 180 mg/L (start – 24 h): 3.1
Dissolved oxygen:	6.2 – 9.0 mg/L
Conductivity:	100 µS/cm
Hardness:	16.0 mg $CaCO_3$/L.
Methods:	The acute toxicity test was performed at nominal concentrations of 10, 18, 32, 56, 100 and 180 mg test item/L prepared using filtered and dechlorinated tap water treated with ultra violet steriliser. The test was conducted under static test conditions (no media renewal). A negative control group (dilution water only) was also prepared. A single vessel was

	prepared for the control and each test media group, each containing ten fish (27.5 L borosilicate glass vessels containing 20 L test medium). Observations: All fish were observed for sublethal effects and mortality after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water was measured at test initiation. Samples of test media were analysed for glyphosate acid content using HPLC analysis at test initiation and after 48 and 96 hours. Statistical calculations: The 96 hour LC50 values and 95 % confidence intervals were calculated using non-linear interpolation. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

The measured concentrations of glyphosate acid in fresh media at test initiation ranged between 96.9 and 110 % of nominal. In aged test media at 96 hours, mean measured glyphosate acid concentrations ranged between 94.4 and 97.0 % of nominal. At 100 and 180 mg/L, no chemical analysis was performed at 48 and 96 hours, as all fish died within the first 24 hours following addition. As measured concentrations of glyphosate acid were between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

There were no mortalities in the control or the 10, 18 and 32 mg /L treatments. At 56 mg test item/L, there was 90 % mortality. There was 100 % mortality at 100 mg/L and higher test concentrations that occurred after 24 hours. There was a strong negative correlation between pH value and test item concentrations observed. At 56 mg test item/L, the pH was reduced to 3.8 and lower.

Table 56: Effects of glyphosate acid on Bluegill sunfish (Lepomis macrochirus)

Glyphosate acid (mg/L)	**% of dead fish and observed symptoms**			
	24 h	**48 h**	**72 h**	**96 h**
Control	< 10	< 10	< 10	< 10
10	< 10	< 10	< 10	< 10
18	< 10	< 10	< 10	< 10
32	< 10	< 10	< 10	< 10
56	40	80	90	90
100	100	100	100	100
180	100	100	100	100

RMS Conclusions

The 96 hour LC_{50} value for bluegill sunfish (*Lepomis macrochirus)* exposed to glyphosate acid was 47 mg glyphosate acid/L (nominal) with a 95 % confidence interval of 35 to 66 mg/L, with a 96 hour NOEC values of 32 mg glyphosate acid/L. The study is considered to be acceptable and valid.

5.4.1.2 Long-term toxicity to fish

Study 1

Author:	Dias Correa Tavares, C.M.
Title:	Chronic Toxicity of Glifosate Técnico Nufarm to zebra fish larvae (*Brachydanio rerio*)
Date:	13.01.2000
Doc ID:	2310938 /RF-D62.16/99
Guidelines:	OECD 212/ IBAMA 1990: Manual de testes para avaliacao da ecotoxicidade de agentes quimicos
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	954.9 g/kg acid equivalent
2. Vehicle and/or positive control:	Tap water; Potassium dichromate ($K_2Cr_2O_7$)
Species:	Zebra fish (*Danio rerio*) larvae
Age:	Larvae, approx. 48 hours old
Size:	Not stated
Loading:	1 L for 10 larvae
Source:	Eggs: in-house. Matrix fish: Peixe Vivo Aquicultura Ltda, Muriae, Brasil
Acclimation period:	48 hours prior to testing during embryo incubation and hatching
Temperature:	23.8-24.3 °C
Photoperiod:	16 hours light / 8 hours dark
Dissolved oxygen:	60-100%
Conductivity:	168 µS/cm
Hardness of test medium:	44.1 mg/L $CaCO_3$
Methods:	The fish early life-stage toxicity test was performed under semi-static exposure conditions renewing the test solution every 48 hours. Following a range finding test, the freshly hatched fry of *Danio rerio* was exposed to test concentrations of 0.32, 0.56, 1.0, 3.2, 5.6, 10 and 32 mg glyphosate acid/L for 168 hours. A control consisting of reconstituted water and five toxic reference concentrations (32, 56, 100, 140 and 180 mg $K_2Cr_2O_7$/L were maintained concurrently. Observations for mortality and sublethal responses were made every 24 hours. Dead individuals were removed at each observation. Temperature, dissolved oxygen, pH and conductivity were measured daily. The active ingredient analysis of stock solutions was performed by liquid chromatography. LC_{50} and its confidence limits were determined using trimmed Spearman-Karber method. Fisher's Exact test was used for determination of significant differences in survival between control and exposure.

Results

The active ingredient concentration in each stock solution was at least 80 % of the nominal concentration. For the reference compound potassium dichromate ($K_2Cr_2O_7$) a 168 hour LC_{50} value of 124.66 mg a.s./L (95 % C.I. 112.08 – 138.67 mg a.s./L) was determined.

With regard to the validity criteria of the pertaining OECD guideline 212 survival of fertilised eggs on successive days was 100 %. Analysis of test item treatments was performed for the stock solutions, the test was carried out in a semi-static system, with renewal of the test solution each 48 h. The water temperature did not differ more than +/- 1.5 °C between test chambers on successive days at any time during the test at the recommended temperature, as well as pH remained constant. Mortality in control group did not exceed 10 %, dissolved oxygen concentration was between 60 and 100 % of air saturation. The present study is considered valid according to OECD guideline 212.

A significant increase of mortality was observed at a concentration of 5.6, 10 and 32 mg a.s./L, behavioural responses such as lethargy was observed at 3.2, 5.6, 10 and 32 mg a.s./L. The following observations for mortality were made every 24 h during the 168 h test period:

Table 57: Lethal effects of glyphosate acid for zebra fish

	Glyphosate acid (mg a.s./L)							
	0 (Control)	**0.32**	**0.56**	**1.0**	**3.2**	**5.6**	**10**	**32**
Introduced	30	30	30	30	30	30	30	30
Survided (168 h)	30	30	30	30	27	25	22	13
Mortality (168 h) (%)	0	0	0	0	10	16.7*	26.7*	56.7*

*statistically significant different from control

RMS Conclusions

In the guideline OECD 212 it is recommended that the duration of the test should be 30 days post hatch. By contrast, the present study was performed for 168 h. It is also stated that the test is to be continued at least until all the fish in control treatment are free feeding. Moreover, the time of first feeding should start 6-7 days after spawning. In the current test it is not clear, if fish in the control treatment are free feeding totally. Nevertheless, significant increase of mortality was observed at a concentration of 5.6, 10 and 32 mg a.s./L. Despite these deficiencies, the study is considered to be valid and acceptable.

In the short term toxicity test on fish larvae, the LC_{50} after 168 hours was determined to be 24.71 mg a.s./L. The No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) for zebra fish (Danio rerio) exposed to glyphosate acid were determined by the author to be 3.2 mg a.s./L and 5.6 mg a.s./L, respectively, based on nominal concentrations. Nevertheless, the mortality effect in the study with *Danio rerio* followed a dose response relationship and in the treatment level at 3.2 mg/L a mortality of 10% was observed. Considering these biological effects as relevant, although not statistically significant, results in a NOEC of 1.0 mg/L.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Author:	Wüthrich, V.
Title:	48-Hour Acute toxicity of Glyphosate techn. to *Daphnia magna* (OECD-Immobilisation Test)
Date:	09.11.1990
Doc ID:	2310947 /272968
Guidelines:	OECD 202 (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9 %
Positive control:	Reconstituted water (EEC), Potassium dichromate ($K_2Cr_2O_7$)
Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 20 mL test medium
Source:	In-house culture
Diet/Food:	Not fed during test or during the 24 hours preceding test initiation.
Acclimation period:	Approximately 24 hours
Temperature:	21.0 ± 0.5 °C
Photoperiod:	16 hours light
pH:	Control:8.4 – 7.9 62.5 mg test item/L:6.3 – 7.6 125 mg test item/L:4.8 – 5.2 250 mg test item/L:3.2 – 3.4 500 mg test item/L: 2.7 – 2.9 1000 mg test item/L:2.3 – 2.6
Dissolved oxygen:	8.3 – 8.1 mg O_2/L (mean)
Conductivity:	Not stated
Hardness:	250 mg $CaC0_3$/L(reconstituted water)
Methods:	The toxicity test was performed with five test nominal glyphosate acid concentrations of 62.5, 125, 250, 500 and 1000 mg glyphosate acid/L, prepared using reconstituted water (EEC). The test was conducted using a static test design (without media renewal) over 48 hours, in duplicate 50 mL beakers each containing 20 mL of the appropriate test or control (reconstituted water only) solution. Juvenile Daphnid (<24 hours old) were added impartially to the test vessels until all contained 10 daphnia. In addition, a test item stability control without daphnids was also prepared at 1000 mg glyphosate acid/L.

	The number of immobile *Daphnia magna* in each vessel was recorded at 24 h and 48 h after test initiation. The pH-values and oxygen saturation were measured in each test vessel at test initiation and termination. Samples of control and test media were taken at the start – 0 hours (freshly prepared – before animal addition) and end – 48 hours (pooled replicates according to treatment) and analysed for glyphosate content using an HPLC method of analysis. The EC_{50} (immobilisation) was estimated by the authores using the Logit-model, NOEC, EC_{50} and EC_{100} values were determined by linear regression. EC_{50} values were recalculate by RMS via ToXRatPro Version 2.10 using Probit analysis using linear max. likelihood regression and Multiple testing to find the NOEC (Bonferroni-Fisher Test).

Results

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L. Measured concentrations of glyphosate acid in the test media at 62.5, 125, 250 and 500 mg glyphosate acid/L were in the range of 69.7 – 95.2 % of nominal. Authors reported results based on nominal glyphosate acid concentrations. According to the actual criteria in this case results should be based on measured concentrations. Therefore endpoints were recalculated by RMS. Results of the probit analysis using linear max. likelihood regression proposed an EC_{50} value of 74.0 (95 % CL: 16.96 - 130.34). A NOEC of 53.2 mg glyphosate/L is calculated.

The pH in test medium was decreasing due to increasing test concentrations, as the test item is an acid.

Immobilisation of daphnids was observed beginning with 62.5 mg/L test item and all daphnids were immobilised after 48 h at the next higher concentration of 125 mg/L test item.

Table 58: Effects of glyphosate on *Daphnia magna*

		Glyphosate acid (mg/L)									
	Control	**62.5**		**125**		**250**		**500**		**1000**	
Mean measured concentrations (mg/L) (% nominal)	-	**53.2 (85)**		**97.6 (78)**		**232.3 (93)**		**475.1 (95)**		**775.2 (78)**	
% immobile daphnids after 24 h	0	10	0	30	60	100	100	100	100	100	100
% immobile daphnids after 48 h	0	10	0	100	100	100	100	100	100	100	100
pH after 24 h	8.4	6.3		4.8		3.2		2.7		2.3	
pH after 48 h	7.9	7.6		5.2		3.4		2.9		2.6	

RMS Conclusions

The authors concluded that the 48 hour EC_{50} (immobilisation) value for *Daphnia magna* exposed to glyphosate acid was 84.0 mg glyphosate/L with a 95 % CL of 73.3 to 110.1 mg/L. The 48 hour NOEC value was 60.3 mg glyphosate /L based on nominal concentrations.

These values were recalculated by the RMS. Results of the probit analysis using linear max. likelihood regression proposed and EC_{50} value of 74 mg/L (95 % CL: 16.966 - 130.338). A NOEC of 53 mg glyphosate/L is suggested by the program.

The study is considered to be acceptable and valid. Nevertheless to address actual criteria recalculation of the endpoints was necessary.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Author:	Magor, S.E., Shillabeer, N.
Title:	Glyphosate acid: Chronic toxicity to *Daphnia magna*
Date:	29.06.1999
Doc ID:	2310962 /BL6535/B
Guidelines:	OECD 202, Part II, Reproduction Test (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P30
Purity:	97.6 %
2. Vehicle and/or positive control:	Elendt M4
Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	1 organism per vessel (glass beakers containing 80 mL test solution)
Source:	Continuous laboratory cultures
Temperature:	19.4 to 20.2 °C
pH:	3.67-8.02 (new solutions) ; 3.46-8.00 (old solutions)
Dissolved oxygen:	9.2-9.2 mg O_2/L (dilution water, new); 8.8-9.2 mg O_2/L (test solutions, old)
Conductivity:	572-617 mg/L µS/cm (test solutions)
Hardness:	202.7-218.3 mg $CaCO_3$
Photoperiod:	16 hours light /8 hours dark, 20 minute dawn and dusk transition period; 480 lux
Methods:	The lethal and sub lethal effects of glyphosate acid on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. Ten replicates of one daphnia per concentration were exposed to 12.5, 25, 50, 100, and 200 mg a.s./L nominal concentrations. In addition, 10 x 1 daphnia were exposed to test medium without test substance (blank control). The daphnia were randomly placed into the test beaker and exposed to the test item for 21 days. The test daphnia were fed daily with cultured algae (*Chlorella vulgaris*). A primary stock solution of 200 mg a.s./L was prepared on day 0 by dissolving 400 mg test item in 2000 mL of dilution water. On days 2, 4, 7, 9, 11, 14, 16, and 18 a primary stock solution of 100 mg a.s./L was prepared by dissolving 200 mg test item in 2000 mL dilution water. The test solutions were prepared by the addition of appropriate aliquots of the stock solutions to dilution water. At each renewal of the test solutions, the surviving P0 generation of daphnia were transferred to the new solutions. The F1 generation of daphnia were removed from each vessel and counted. The numbers of alive and dead F1daphnia were recorded. Mortality of P0 generation of daphnia and observation for the presence of alive and dead offspring (termed F1 generation) were recorded daily in each test vessel. At the end of the test, the length of each surviving P0 daphnia was measured. The pH was measured in each newly prepared test solution. The pH and dissolved oxygen concentration of two of the replicates of the old test solutions were measured after transfer

	of the P0 generation of daphnids. Temperature measurements were recorded daily by means of a thermometer and hourly automatically. The concentration of glyphosate acid in the test solutions was determined on days 0, 2, 7, 9, 14, and 16. Old solutions were analysed on days 2, 7, 9, 14, and 21. The reproduction and length data for each individual P0 generation daphnid were entered into electronic data files and analysed using statistical procedures contained in the Brixham Environmental Laboratory computer programs 'STATS' (version 4.10) and 'EPA' (version1.04).

Results

The validity criteria according to OECD 202 were fulfilled, as immobility of daphnids was < 20 % in control groups and mean offspring number at day 21 was > 60.

The effects of glyphosate acid on *Daphnia magna* mortality and reproduction are shown in the following table.

Table 59: Offspring per day and female of *Daphnia magna*

Nominal concentration (mg a.s./L)	Mean adult mortality (%)	Total offsprings per parent (No.)	Total offsprings (No.)
Control	10	108± 20	1028
12.5	0	100±21	1003
25	0	84±12*	840
50	0	91±18	912
100	50	105±23	763

* Statistically significant difference

At the nominal concentration of 25 mg/L the total number of offspring per parent was significantly lower when compared to control. Even though the results of this study do not show a classical dose response relation, significant effects were observed and it is proposed to consider these effects. The relevant and accepted long term endpoint for invertebrates established in the EU evaluation of glyphosate in 2001 is in the same order of magnitude.

RMS Conclusions

The study was performed according to OECD 202, Part II. According to current criteria, the OECD 211 would be the relevant directive. Since daphnids were held individually in the test vessel, it is possible to determine the exact number of offspring per parent and therefore a statistical evaluation according to the criteria of OECD 211 is possible. RMS proposes to consider significant effects at 25 mg/L and recommends an NOEC for reproduction 12.5 mg a.s./L based on nominal concentration.

The overall 21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid is 12.5 mg a.s./L based on nominal concentration.

5.4.3 Algae and aquatic plants

Study 1

Author:	Smyth, D.V., Kent, S.J., Morris, D.S., Shearing, J.M., Shillabeer, N.
Title:	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum*
Date:	08.11.1996
Doc ID:	2310972 /BL5684/B
Guidelines:	OECD 201 (1984), US EPA Guideline 540/09-82-020 (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item::	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
Cell growth medium	Cell growth medium (Walsh & Alexander 1980)
Species:	Marine alga *Skeletonema costatum*, strain CCAP 1077/1C
Source:	Culture centre of algae and protozoa, Dunstaffnage Marine Laboratory, Oban, Argyll, UK
Initial cell concentration	1.00 x 10^4 cells/mL
Temperature:	20.0-20.1°C (measured by thermometer). The hourly temperature measured automatically remained within 20±1 °C.
Photoperiod:	16 h light
Light intensity:	4340 lux
pH:	7.1 – 8.1 at the start of the test, 8.1 – 8.8 at the end of the test
Methods:	The toxicity of glyphosate acid to the marine alga *Skeletonema costatum* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.s./L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution. A stock solution of nominal concentration of 56 mg a.s./L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18, and 32 mg a.s./L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel. The test was performed in 6 replicates cultures for control and 3 replicates for each concentration of glyphosate acid. Each replicate was inoculated with 1.250 mL of the inoculum culture to give a nominal cell density of 1.00 x 10^4 cells/mL. The culture vessels were incubated at 20±1°C for 120 h. During incubation, the cells were kept in suspension by continuous shaking. The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system. The

	concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test. One-way analysis of variance, and Dunnett's procedure. Median effective concentrations and its 95% confidence limits were determined by linear regression against log concentration.

Results

The biomass in the control cultures increased by a factor of > 16, the coefficient of variance for section specific growth rates was ≤ 35 %, for the whole test period it was ≤ 7 %. The validity criteria according to guideline OECD 201 were therefore fulfilled.

The mean measured concentrations of glyphosate acid ranged from 94 to 106 % of the nominal values. On the basis of the analytical results being with 80 and 120 % of the nominal test concentration, ecotoxicological endpoints were evaluated using the nominal concentrations.

Table 60: Mean cell densities and percentage of inhibition of cell growth of *Skeletonema costatum* exposed for 72 and 96 hours to glyphosate

Nominal concentration (mg a.s./L)	**Mean growth rates 72h**		**Mean areas under the growth curve 72h**		**Mean growth rates 96h**		**Mean areas under the growth curve 96h**	
	Mean growth rate	**% of control**	**Mean areas under the growth curve**	**% of control**	**Mean growth rate**	**% of control**	**Mean areas under the growth curve**	**% of control**
Control	1.423		37.4		1.113		97.6	
1.0	1.423	101	38.0	102	1.112	100	99.0	101
1.8	1.433	101	38.9	104	1.113	100	100.8	103
3.2	1.443	93	29.5*	79	1.128	101	84.5	87
5.6	1.322*	97	34.2	92	1.121	101	92.6	95
10.0	1.387	78	17.9*	48	1.122	101	62.6	64
18.0	1.111*	25	2.8*	8	0.317*	28	4.6	5
32.0	0.362*	21	2.3*	6	0.190*	17	3.3	3
56.0	0.295*	13	1.5*	4	0.087*	8	1.9	2

* Significant difference from the culture control (α =0.05)

RMS Conclusions

The 72 h E_bC_{50} for *Skeletonema costatum* exposed to glyphosate acid was 11 mg/L (95 % C.I. 7.1 to 20 mg a.s./L) and the 96 h E_bC_{50} was 11 mg/L (95 % C.I. 7.2 to 19 mg a.s./L); the 72 h E_rC_{50} was 18 mg/L (95 % C.I. 10 to 42 mg a.s./L) and the 96 h E_rC_{50} was 29 mg/L (95 % C.I. 16 to > 56 mg a.s./L) (nominal). The 72-hour NOE_bC and NOE_rC values were 1.82 mg/L (nominal), respectively.

The study is considered to be valid and acceptable.

Study 2

Author:	Smyth, D.V., Shillabeer, N., Morris, D.S., Wallace, S.J.
Title:	Glyphosate acid: Toxicity to blue-green alga *Anabaena flos-aquae*
Date:	08.11.1996
Doc ID:	2310970 /BL5698/B
Guidelines:	OECD 201 (1984), US EPA Guideline 540/09-82-020 (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
Medium	acc. to Miller et al. (1978)
Species:	Blue-green alga *Anabaena flos-aquae*
Source:	Brixham Environmental Laboratory culture from strain CCAP 1403/13A, Culture Centre of Algae and Protozoa, Institute of Freshwater Ecology. Windermere Laboratory, Far Sawrey, Ambleside, Cumbria, UK
Initial cell concentration	2.05 x 10^4 cells/mL
Temperature:	24.1-24.2 °C (measured by thermometer) The hourly temperature measured automatically remained within 24±1°C
Photoperiod:	Continuous illumination
Light intensity:	3600 lux
pH:	3.5 – 7.2 at the start of the test, 3.6 – 8.2at the end of the test
Methods:	The toxicity of glyphosate acid to Anabaena flos-aquae was determined in a 120-hour, static toxicity test. The test incorporated 8 nominal concentrations of glyphosate acid (0.75, 1.5, 3.0, 6.0, 12, 24, 48, 96 mg a.s./L) and a negative control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution. A stock solution at a nominal concentration of 96 mg glyphosate/L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 0.75, 1.5, 3.0, 6.0, 12, 24, and 48 mg a.s./L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel. The test was performed in 6 replicates for the control group and 3 replicates for each concentration of glyphosate acid. Each replicate was inoculated with 1.120 mL of the inoculum culture to give a nominal cell density of 2.05 x 104 cells/mL. Single blank vessels were prepared for the control and each test concentration without algal cells. The culture vessels were incubated at 24±1°C under continuous illumination for 120 h. During incubation, the algal cells were kept in suspension by continuous shaking. The algal cell densities were determined by spectrophotometric absorbance, using a Uvikon 860 UV/visible spectrophotometer. After 1, 2, 3, 4, and 5 days, samples were removed from each control, test and blank vessel. The appropriate blank solution absorbance was subtracted from that of the test culture to obtain the algal absorbance reading. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily and hourly. The

	concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test. One-way analysis of variance, and Dunnett's procedure. Median effective concentrations and its 95% confidence limits were determined by linear regression against log concentration.

Results

The biomass in the control cultures increased by a factor of > 16, the coefficient of variance for section specific growth rates was ≤ 35 %, for the whole test period it was ≤ 7 %. The validity criteria according to guideline OECD 201 are therefore fulfilled.

The mean measured concentrations of glyphosate acid ranged from 98 to 110 % of the nominal values. On the basis of the analytical results being with 80 and 120 % of the nominal test concentration, ecotoxicological endpoints were evaluated using the nominal concentrations.

Table 61: Mean growth rates and mean areas under the growth curve of *Anabaena flos-aquae* exposed for 72 and 96 hours to glyphosate acid

Nominal concentration (mg a.s./L)	**Mean growth rates 72h**		**Mean areas under the growth curve 72h**		**Mean growth rates 96h**		**Mean areas under the growth curve 96h**	
	Mean growth rate	**% of control**	**Mean areas under the growth curve**	**% of control**	**Mean growth rate**	**% of control**	**Mean areas under the growth curve**	**% of control**
Control	1.392	-	1.331	-	1.331		1.5	-
0.75	1.365	91	1.357	98	1.357	102	1.5	103
1.5	1.336	85	1.355	96	1.355	102	1.5	99
3.0	1.328	80	1.344	95	1.344	101	1.4	94
6.0	1.321	82	1.342	95	1.342	101	1.4	94
12	1.299	76	1.321	93	1.321	99	1.3	87
24	1.231*	6	0.216*	17	0.216*	16	0.0*	2
48	0.231*	5	0.173*	17	0.173*	13	0.0*	2
96	0.231*	5	0.173*	17	0.173*	13	0.0*	2

* Significant difference from the culture control (α =0.05)

RMS Conclusions

The 72 h E_bC_{50} for *Anabaena flos-aquae* exposed to glyphosate acid was 8.5 mg a.s./L (95 % CL 2.6 to 28 mg a.s./L), the 72 h E_rC_{50} was 22 mg/L (95 % CL 8.8 to >96 mg a.s./L) and the 72-hour NOE_bC and NOE_rC values were 12 mg/L (nominal), respectively.

The study is considered to be valid and acceptable.

Study 3

Author:	Smyth, D.V., Kent, S.J., Morris, D.S., Cornish, S.K., Shillabeer, N
Title:	GLYPHOSATE ACID: Toxicity to duckweed (*Lemna gibba*)
Date:	31.01.1996
Doc ID:	2310988 /BL5662/B
Guidelines:	EPA FIFRA Subdivision J Guideline 123-2
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid	
Description:	White solid	
Lot/Batch #:	P24	
Purity:	95.6 %	
2. Vehicle and/or positive control:	Hoaglands M medium	
Species:	*Lemna gibba*, Strain G3	
Source:	In-house culture originally obtained from University of Waterloo, Canada	
Temperature:	24.6 – 25.0 °C	
Photoperiod:	24 h illumination	
Light intensity	5000 lux	
pH:	Freshly prepared test media: Control:4.7 – 4.9 0.75 mg/L: 4.7 – 4.8 1.5 mg/: 4.6 – 4.7 3.0 mg/L: 4.6 6.0 mg/L: 4.5 12 mg/L: 4.4 24 mg/L: 4.2 – 4.3 48 mg/L: 3.9 – 4.0 96 mg/: 3.5 – 3.6	Old test media: Control:5.3 – 5.7 0.75 mg/L: 5.3 – 5.8 1.5 mg/: 5.2 – 5.8 3.0 mg/L: 5.2 – 5.8 6.0 mg/L: 5.1 – 5.7 12 mg/L: 4.8 – 5.6 24 mg/L: 4.6 – 5.0 48 mg/L: 4.0 – 4.2 96 mg/: 3.6 – 3.7
Methods	The toxicity test on *Lemna gibba* was performed with eight concentration levels, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg glyphosate acid/L with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions as the test groups. The plants were placed in 400 mL beakers (test vessels), containing 160 mL of Hoagland's M-medium prepared according to Hillman (1961). The test was conducted under semi-static conditions with renewal of the test medium after 5 and 9 days. Three uniform healthy-looking plants with 4 fronds each were added to each control and test vessel. The number of plants and fronds were counted after 2, 5, 7, 9, 12 and 14 days. Also symptoms of toxicity were recorded on these dates. At test end the weight of the dried plant tissue (at 60 °C) was recorded. The pH was measured in the old and the new test medium (new= day 0, 5 and 9, old = day 5, 9 and 14). Temperature in the test chamber was recorded daily and light intensity was recorded once a week. Analytical measurements of glyphosate acid were performed by means of HPLC analysis at test start and after 5 and 9 d (after test medium renewal). Fresh media was analysed on days 0, 5 and 9. Old media were analysed on days 5, 9 and 14.	

	The EC_{50} and its 95% confidence interval were calculated by moving average angle method. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dry weight, respectively, at p = 0.05.

Results

Analytical measurements were performed in the freshly prepared (day 0, 5 and 9) and the old (day 5, 9 and 14) test media. The measured concentrations in the fresh media ranged from 90 – 108 % of nominal and in the old media from 87 – 102 % of nominal (overall mean measured: 93 – 100 % of nominal).

All validity criteria according to OECD 221 were fulfilled, as the doubling time of frond numbers in the control were less than 2.4/d. According to EPA FIFRA Subdivision J Guideline 123-2, endpoints were determined after 14 days.

The increase in frond number was significantly inhibited at nominal test concentration of 6.0 mg test item/L and higher, when compared to the control. The growth of the plant in terms of tissue dry weight was significantly reduced at 12 mg test item/L and higher. At 24, 48 and 96 mg test item/L dose related symptoms like pale frond colouration, emergence of stunted new frond growth, reduced root growth and unnatural floating on the solution surface were observed from day 2 onwards. Visually observed effects were apparent at concentrations of 3.0 mg/L and above.

Table 62: Frond numbers, increase in frond numbers and inhibition compared to the control

Test item rate (mg a.s./L)	Number of fronds						Increase in frond numbers	Inhibition (%)
	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	(Day 0 – 14)	
Control	21	48	85	134	222	327	315	-
0.75	23	47	79	125	232	343	331	0
1.5	23	45	78	113	220	323	311	1
3.0	21	48	78	120	206	300	288	9
6.0	21	49	81	116	198	269	257	18*
12	20	44	74	105	148	173	161	49*
24	16	28	44	59	82	91	79	75*
48	15	21	24	28	28	30	18	94*
96	13	14	15	16	18	17	5	98*

* significant at p = 0.05

Table 63: Mean dry weight of plant tissue after 14 d, main increase in dry weight and inhibition compared to the control

Test item rate (mg a.s./L)	Mean tissue dry weight after 14 day (mg)	Mean increase (mg)	Inhibition (%)
Control	40.7	39.2	-
0.75	51.3	49.8	0
1.5	49.8	48.3	0
3.0	44.0	42.5	0
6.0	40.3	38.8	1
12	29.8	28.3	28*
24	16.5	15.0	62*
48	6.0	4.5	89*
96	1.4	> 0.1	100*

* significant at p = 0.05

RMS Conclusions

Glyphosate acid was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 6 mg a.s./L. The 14-d EC_{50} value for inhibition of frond number was 12 mg a.s./L (95% CL = 11 - 14 mg a.s./L) and for tissue dry weight 20 mg a.s./L (95% CL = 18 – 22 mg a.s./L).The NOEC was determined to be 3.0 and 6.0 mg a.s./L for frond number and weight increase, respectively.

The study is considered to be valid and acceptable

5.4.4 Other aquatic organisms (including sediment)

No data available.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Glyphosat produces acute $L(E)C_{50}$ values in concentrations 18 - 22 mg/L for algae, 12 mg/L for aquatic plants, 84 mg/L for crustaceans and 47 mg/L for fish. Chronic NOEC values in concentrations of > 1 mg/L for algae and aquatic plants, > 10 mg/L for invertebrates and 1 mg/L for fish were determined.

The results of the test on the biodegradation of glyphosat in the water/sediment system show that glyphosat is considered not rapidly degradable (a degradation > 70 % within 28 days) for purposes of classification and labelling.

Glyphosat has a log $K_{o/w}$ of – 3.2. The experimentally derived kinetic BCF of 1.1 for glyphosat related to total radioactivity, whole fish is lower than the trigger of 500 (criterion for bioaccumulation potential conform Regulation EC 1272/2008).

CLP- Acute aquatic hazards

According to the criteria of the CLP Regulation, a substance is classified for aquatic acute toxicity if in an aquatic acute toxicity study, an $L(E)C_{50}$ of ≤ 1 mg/l is obtained for any of the three trophic levels fish, invertebrates and algae/aquatic plants.

The lowest $L(E)C_{50}$ obtained for glyphosat are 18, 12, 84 and 47 mg/L in algae, aquatic plants, invertebrates and fish, respectively. Glyphosat therefore do not fulfil the criteria for classification as Aquatic Acute Cat. 1.

CLP - Aquatic chronic hazards

According to the criteria of the 2nd ATP to the CLP Regulation, when NOEC values are available for all trophic levels, a substance is classified for aquatic chronic hazards if a NOEC or EC_{10} of ≤ 1 mg/L is obtained in a long-term aquatic toxicity study. The assignment of a hazard category depends on the NOEC value and whether the substance is rapidly degradable or not.

Glyphosat is considered not rapidly degradable (see section 5.1.3). NOEC values for glyphosat are available for all trophic levels. The lowest NOEC is 1 mg/L obtained for fish. Glyphosat therefore fulfils criteria for classification as Aquatic Chronic Cat. 2.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Glyphosat fulfils the criteria for classification as Aquatic Chronic 2.

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, glyphospate 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 poptential 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 (*Lepomis macrochirus*) OECD TG 203/FIFRA 72-1 Static exposure	LC_{50} (96h) = 47 mg/L (nom)	-	Kent *et al.* (1995)
zebra fish larvae (D*anio rerio*) 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 *Daphnia magna* OECD TG 202 static exposure	LC_{50} (48 h) = 84 mg/L (nom) 74 mg/L (meas)		Wüthrich (1990)
Acute toxicity to *Daphnia magna* 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 *Skeletonema costatum* OECD TG 201 static exposure	ErC_{50} (72 h) = 18 mg/L (nom)	NOErC (72 h) = 1.82 mg/L (nom)	Smyth *et al.* (1996)
blue-green alga *Anabaena flos-aquae* OECD TG 201 static exposure	ErC_{50} (72 h) = 22 mg/L (nom)	NOErC (72 h) = 12 mg/L (nom)	Smyth *et al.* (1996)

duckweed (*Lemna gibba*) EPA FIFRA Guideline 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 *et al.* (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 termined 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 critera 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.

Additional key elements

Water-sediment tests

It is presented in the RAR that the valid water/sediment studies show, in addition to microbial degradation, that a major contributor to the aquatic dissipation of glyphosate is adsorption to sediment. The dissipation times (DT_{50}) are presented in the table below. Approximately, 6% to 48% of the applied glyphosate is mineralised to carbon dioxide (CO_2) during 91 or 100 days of incubation. Radioactivity associated with non-extractable residues was between 8% and 35% of the applied glyphosate during 97 or 91 days of incubation. The principal degradant of glyphosate in the water/sediment system is AMPA (aminomethyphosphonic acid). The maximum amount of AMPA detected was 16% (water phase), 19% (sediment) and up to 27% (total system) of the total glyphosate applied. The degradant HMPA (hydroxymethylphosphonic acid) was only detected in the water phase at 10 %.

Re-calculated (FOCUS) DT_{50} values from relevant water/sediment studies

	DT_{50} (total system)	**DT_{50} (water)**	**DT_{50} (sediment)**
Glyphosate	13.8-329.9 days	6.8-21.8 days	34.1- 75.6 days
AMPA	69.3-102.9 days	2.1-15.5 days	-

Aquatic toxicity of AMPA
Acute

Species	**Test design**	**EC_{50} (mg a.s./L)**	**NOEC (mg a.s./L)**
Oncorhynchus mykiss	96 h static	520	32
Daphnia magna	48 h static	690	320
Pseudokirchneriella subcapitata	72 h static	ErC_{50} 200	-

Long-term

Species	**Test design**	**EC_{50} (mg a.s./L)**	**NOEC (mg a.s./L)**
Pimephales promelas	33 day (7 days post-harch)	-	12
Daphnia magna	21 d semi-static	90	15
Pseudokirchneriella subcapitata	72 h static	-	NOErC: 46
Myriophyllum aquaticum	14 d static	72.0 dry weight, growth rate	NOEC <5.4

Aquatic toxicity of HMPA

Species	**Test design**	**EC_{50} (mg a.s./L)**	**NOEC (mg a.s./L)**
Daphnia magna	48 h static	>100	100
Pseudokirchneriella subcapitata	72 h static	ErC_{50} >115	NOAEC: 60
Lemna gibba	7 d semistatic	EC_{50}, frond count >123 EC_{50}, dry weight >123	NOEC = 123

Aquatic toxicity

In the RAR, additional tests are reported for each trophic level that support the environmental classification as Aquatic Chronic 2.
In particular, the lowest acute and chronic aquatic toxicity data refer to the aquatic plant *Myriophyllum aquaticum* (Wenzel, 2012). The test was performed following Maltby *et al*. (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP. Over 14 days,

fresh weight was found to be the most sensitive parameter. The 14 days EC_{50} value for fresh weight inhibition was 4.4 mg glyphosate acid equivalents/L (mean measured). At the lowest tested concentration (0.3 mg/L) an inhibition of 20.7% in the fresh weight increase and an inhibition of 14.6% in the fresh weight growth rate were observed. Therefore, the NOEC for both fresh weight parameters is < 0.3 mg glyphosate acid equivalents/L (based on geometric mean measured concentrations).

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 DT_{50} 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 leves (fish, invertebrates and algae/aquatic plants). The lowest $L(E)C_{50}$ 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, $L(E)C_{50} \leq 1$ 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.

RAC evaluation of hazards to the ozone layer

This hazard class was not evaluated.

6 OTHER INFORMATION

None

7 REFERENCES

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61	Chruscielska, K.; Brzezinski, J.; Kita, K. et al.	2000	Glyphosate: Evaluation of chronic activity and possible far - reaching effects - Part 1. Studies on chronic toxicity Page: 11-19 Pestycydy, 2000, (3-4), 11-20. GLP: No Published: Yes BVL-2716174, ASB2013-9829	No	LIT
62	Clay, P.	1996	Glyphosate acid: L5178Y TK+/- mouse lymphoma mutation assay CTL/P/4991 ! VV 0123 BVL-2154316, TOX2000-1994		SYD SYN
63	Cocco, P.; Satta, G.; Dubois, S.; Pili, C.; Pilleri, M.; Zucca, M.; Martine 't Mannetje, A.; Becker, N.; Benavente, Y.; de Sanjosé, S.; Foretova, L.; Staines, A.; Maynadié, M.; Nieters, A.; Brennan, P.; Miligi, L.; Ennas, M. G.; Boffetta, P.;	2012	Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study page 91-98 Occup Environ Med 2012;0:1–7 GLP: No Published: Yes BVL-2716321, ASB2014-7523	No	LIT
64	Coles, L.J., Thomas, O.N., Bartlett, A.J., Brooks, P.N	1996	Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study In The Rat 434/016 GLP: Yes Published: No BVL-2309256, ASB2012-11451	Yes	NUF
65	Coles, R.J., Doleman, N.	1996	Glyphosate technical: Oral gavage teratology study in the rabbit 434/020 GLP: Yes Published: No BVL-2309448, ASB2012-11499	Yes	NUF
66	Colvin, L. B.; Miller, J. A.	1973	Final report on CP 67573 residue and metabolism. Part 9: The gross distribution of n-phosphonomethylglycine-14C in the rabbit 298 ! 9-23-760.06-7863 BVL-1345067, TOX9552353	Yes	BAY CAD CHE DOW MOD MON MOT NUD
67	Colvin, L. B.; Miller, J. A.	1973	CP 67573 residue and metabolism. Part 13: The dynamics of accumulation and depletion of orally ingested N-phosphonomethylglycine-14C	Yes	BAY CAD CHE DOW MOD

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			309 BVL-1345065, TOX9552355		MON MOT NUD
68	Costa, K. C.	2010	Amendment No. 1 to report: Evaluation of the mutagenic potential of Glyphosate technical by micronucleus assay in mice 3996.402.395.07 BioagriI Laboratorios Ltda. GLP: Yes Published: No BVL-2715988, ASB2014-9284	Yes	Helm
69	Costa, K.C.	2008	Evaluation of the mutagenic potential of Glyphosate technical by micronucleus assay in mice RF - 3996.402.395.07 GLP: Yes Published: No BVL-2309333, ASB2012-11481	Yes	HAG
70	Cuthbert, J. A.; Jackson, D.	1989	Glyphosate technical: Acute dermal toxicity (limit) test in rats 243268/5884 BVL-2309119, TOX9300328	Yes	CHE DOW
71	Cuthbert, J. A.; Jackson, D.	1989	Glyphosate technical: Acute oral toxicity (limit) test in rats 5883 ! IRI 243268 BVL-1344956, TOX9552319	Yes	BAY CAD CHE DOW MOD MOT NUD
72	Cuthbert, J. A.; Jackson, D.	1989	Glyphosate technical: Magnusson-Kligman maximisation test in guinea pigs 5887 ! IRI 243268 BVL-1344980, TOX9552343	Yes	BAY CAD CHE DOW MOD MOT NUD
73	Dallegrave, E., Mantese, F.D., Coelho, R.S., Pereira, J.D., Dalsenter, P.R., Langeloh, A.	2003	The teratogenic potential of the herbicide glyphosate-Roundup (R) in Wistar rats page 45-52 Toxicology Letters 142 (2003) 45-52 GLP: No Published: Yes BVL-2309692, ASB2012-11600		LIT
74	Dallegrave, E.; Mantese, F.D.; Oliveira, R.T.; Andrade A.J.; Dalsenter, P.R.; Langeloh, A.	2007	Glyphosat: Pre-and postnatal toxicity of the commercial glyphosate formulation in Wistar rats page 665-673 Arch Toxicol (2007) 81:665–673 GLP: No Published: Yes BVL-2309694, ASB2012-2721		LIT
75	Davies, D. J.	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat CTL/P/4940 GLP: Open (1) Yes (3) Published: No BVL-2154302, TOX2000-1977	Yes	SYD SYN
76	Davies, D. J.	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat CTL/P/4942 BVL-2154303, TOX2000-1978	Yes	SYD SYN
77	Davies, D. J.	1996	Glyphosat acid: Whole body autoradiography in the rat (10 mg/kg) CTL/P/4943 ! UR 0509 BVL-2154300, TOX2000-1980	Yes	SYD SYN

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78	Davies, D. J.	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat following repeat dosing CTL/P/4944 BVL-2154304, TOX2000-1979	Yes	SYD SYN
79	De Roos, A.J., Blair, A., Rusiecki, J.A., et al.	2005	Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study page 49-54 Environmental Health Perspectives, VOLUME 113, NUMBER 1 GLP: No Published: Yes BVL-2309704, ASB2012-11605	No	LIT
80	De Roos, A.J., Zahm, S.H., Cantor, K.P., Weisenburger, D.D., Holmes, F.F., Burmeister, L.F., Blair, A.	2003	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men Occupational and Environmental Medicine vol.60, 9 (2003) GLP: No Published: Yes BVL-2309706, ASB2012-11606	No	LIT
81	Decker, U.	2007	Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats B02327 GLP: Yes Published: No BVL-2309161, ASB2012-11414	Yes	NUF
82	Dhinsa, N.K., Watson, P., Brooks, P.N	2007	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat 2060/0013 GLP: Yes Published: No BVL-2309418, ASB2012-11494	Yes	NUF
83	Dideriksen, L. H.; Skydsgaard, K.	1991	Assessment of acute oral toxicity of "Glyphosate technical" to mice - incl. Addendum 12321 BVL-1344955, TOX9552320	Yes	BAY CAD CHE DOW MOD MOT NUD
84	Do Amaral Guimaraes, S. P.	2008	Acute oral toxicity study in Wistar Hannover rats for Glyphosate technical RF-3996.305.475.07 GLP: Yes Published: No BVL-2309100, ASB2012-11389	Yes	HAG
85	Do Amaral Guimaraes, S.P.	2008	Acute Dermal Toxicity in Wistar Hannover Rats for Glyphosate Technical RF-3996.310.456.07 GLP: Yes Published: No BVL-2309135, ASB2012-11402	Yes	HAG
86	Doyle, C. E.	1996	Glyphosate acid: Acute oral toxicity study in rats CTL/P/4660 ! AR 5959 BVL-2154305, TOX2000-1982	Yes	SYD SYN
87	Doyle, C. E.	1996	Glyphosate acid: Acute dermale toxicity study in the rats CTL/P/4664 ! CR 3236 BVL-2154306, TOX2000-1983	Yes	SYD SYN
88	Doyle, C. E.	1996	Glyphosate acid: Skin irritation to the rabbit CTL/P/4695 ! EB 4365 BVL-2154308, TOX2000-1985	Yes	SYD SYN

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
89	Doyle, C. E.	1996	Glyphosate acid: Skin sensitisation to the guinea pig CTL/P/4699 ! GG 6427 BVL-2154310, TOX2000-1987	Yes	SYD SYN
90	Durward, R.	2006	Glyphosate Technical: Micronucleus Test In The Mouse 2060/014 GLP: Yes Published: No BVL-2309327, ASB2012-11478	Yes	NUF
91	Eadie, A.; Barrins, C.; Cleere, W. F. et al.	1989	Glyphosate technical: 90 day oral toxicity study in the rats - incl. Amendment to Protocol BY-401 BY-891002 ! BY-401 BVL-2331648, TOX9551821	Yes	BCL
92	EFSA	2012	Final review of the Séralini et al. (2012a) publication on a 2-year rodent feeding study with Glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology EFSA Journal 2012;10(11):2986 ! EFSA-Q-2012-00842 EFSA Journal 2012; 10(11): 2986. vol.10, 11 (2012) 2986-2996 GLP: No Published: Yes BVL-2716077, ASB2012-15513	Yes	LIT
93	EFSA	2015	Peer Review Report on Glyphosate ASB2015-12200		
94	El-Zaemey, S.; Heyworth, J.	2013	Noticing pesticide spray drift from agricultural pesticide application areas and breast cancer: a case-control study Aust NZ J Public Health. 2013 GLP: No Published: Yes BVL-2716417, ASB2014-9473	Yes	LIT
95	Engel, L.S., Hill, D.A., Hoppin, J.A., Lubin, J.H., Lynch, C.F., Pierce, J., Samanic, C., Sandler, D.P., Blair, A., Alavanja, M.C.	2005	Pesticide use and breast cancer risk among farmers' wives in the agricultural health study American Journal of Epidemiology vol.161, 2 (2005) 121-135 GLP: No Published: Yes BVL-2309720, ASB2012-11613	No	MOD
96	Enomoto, A.	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 1 (Seite 1-500) IET 94-0150 Vol.1 GLP: Yes Published: No BVL-2309360, ASB2012-11484	Yes	ALS
97	Eriksson, M., Hardell, L., Carlberg, M., Akerman, M.	2008	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis Int J Cancer vol.123, 7 (2008) 1657-1663 GLP: No Published: Yes BVL-2309722, ASB2012-11614	No	LIT
98	Flower, K.B., Hoppin, J.A., Lynch, C.F., Blair, A., Knott, C., Shore, D.L., Sandler, D.P.	2004	Cancer risk and parental pesticide application in children of agricultural health study participants Environmental Health Perspectives vol.112, 5 (2004) 361-635 GLP: No Published: Yes BVL-2309734, ASB2012-11620	No	LIT

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
99	Flügge, C.	2009	Mutagenicity study of glyphosate TC in the salmonella typhimurium reverse mutation assay (in vitro) LPT 23916 GLP: Yes Published: No BVL-2309303, ASB2012-11468	No	HAG
100	Flügge, C.	2009	Micronucleus Test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration LPT 23917 GLP: Yes Published: No BVL-2309329, ASB2012-11479	Yes	HAG
101	Flügge, C.	2010	Mutagenicity study of Glyphosate TC in the salmonella typhimurium reverse mutation assay (in vitro) LPT 24880 GLP: Yes Published: No BVL-2309305, ASB2012-11469	No	HAG
102	Fox, V.	1998	Glyphosate acid: In vitro cytogenetic assay in human lymphocytes CTL/P/6050 ! SV 0777 BVL-2154314, TOX2000-1995	No	SYD SYN
103	Fox, V.; Mackay, J. M.	1996	Glyphosate acid: Mouse bone marrow micronucleus test CTL/P/4954 ! SM 0796 BVL-2154317, TOX2000-1996	Yes	SYD SYN
104	Freeman,L.B.	2009	Evaluation of agricultural exposures: the agricultural health study and the agricultural cohort consortium Reviews on Environmental Health vol.24, 4 (2009) 311-318 GLP: No Published: Yes BVL-2309740, ASB2012-11623	No	MOD
105	Fritschi, L., Benke, G., Hughes, A.M., Kricker, A..,Turner, J., Vajdic, C.M., Grulich, A., Milliken, S., Kaldor, J., Armstrong, B.K.	2005	Occupational exposure to pesticides and risk of non-Hodgkin's lymphoma American Journal of Epidemiology vol.162, 9 (2005) 849-857 GLP: No Published: Yes BVL-2309746, ASB2012-11624	No	LIT
106	Gaou, I.	2007	Glyphosate Technical: 13-Week Toxicity Study By Oral Route (Capsule) In Beagle Dogs 29646 TCC GLP: Yes Published: No BVL-2309262, ASB2012-11454	Yes	NUF
107	Garry, V.F., Harkins, M.E., Erickson, L.L., Long-Simpson, L.K., Holland, S.E., Burroughs, B.L.	2002	Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA Environmental Health Perspectives 110:441-449 vol.110 (2002) 441-449 GLP: No Published: Yes BVL-2309750, ASB2012-11626	No	LIT
108	George, J., Prasad, S., Mahmood, Z.,	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach J Proteomics vol.73, 5 (2010) 951-964	No	LIT

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	Shukla, Y.		GLP: No Published: Yes BVL-2309766, ASB2012-11829		
109	Germany	1998	glyphosate (Monograph) 11 Dezember 1998 GLP: Open Published: Yes ASB2010-10302	Open	
110	Giknis, M. L. A.; Clifford, C. B.;	2005	Spontaneous neoplastic lesions in the Crl:CD1 (ICR) mouse in control groups from 18 month to 2 year studies ASB2007-5200	Yes	DOW
111	Goburdhun, R.	1990	Glyphosate: 52 week oral toxicity study in dogs 7502 ! IRI 642675 BVL-1344992, TOX9552384	Yes	BAY CAD CHE DOW MOD MOT NUD
112	Goburdhun, R.; Oshodi, R. O.	1989	Glyphosate: Oral maximum tolerated dose study in dogs 5660 ! IRI 640683 BVL-1344982, TOX9552352	Yes	BAY CAD CHE DOW MOD MOT NUD
113	Griffith, D.R.	2009	Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat 2743/0001 GLP: Yes Published: No BVL-2309149, ASB2012-11408	Yes	EXC
114	Haag, V.	2007	Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule)in Beagle Dogs 29647 TCC GLP: Yes Published: No BVL-2309274, ASB2012-11457	Yes	NUF
115	Hadfield, N.	2012	Glyphosate acid - In Vitro Absorption through Abraded Rabbit Skin using [14C]-glyphosate JV2182-REG GLP: Yes Published: No BVL-2309282, ASB2012-11459	No	EGT
116	Haferkorn, J.	2009	Acute oral toxicity study of Glyphosate TC in rats 23910 GLP: Yes Published: No BVL-2309092, ASB2012-11385	Yes	HAG
117	Haferkorn, J.	2009	Acute Inhalation Toxicity Study of Glyphosate TC in Rats LPT 23911 GLP: Yes Published: No BVL-2309151, ASB2012-11409	Yes	HAG
118	Haferkorn, J.	2009	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 23912 GLP: Yes Published: No BVL-2309127, ASB2012-11398	Yes	HAG
119	Haferkorn, J.	2009	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) LPT 23915	Yes	HAG

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			GLP: Yes Published: No BVL-2309231, ASB2012-11443		
120	Haferkorn, J.	2010	Acute oral toxicity study of Glyphosate TC in rats 24602 GLP: Yes Published: No BVL-2309096, ASB2012-11387	Yes	HAG
121	Haferkorn, J.	2010	Acute Inhalation Toxicity Study of Glyphosate TC In Rats 24603 GLP: Yes Published: No BVL-2309145, ASB2012-11406	No	HAG
122	Haferkorn, J.	2010	Acute oral toxicity study of Glyphosate TC in rats 24874 GLP: Yes Published: No BVL-2309094, ASB2012-11386	Yes	HAG
123	Haferkorn, J.	2010	Examination Of Glyphosate TC In The Skin Sensitisation Test In Guinea Pigs According To Magnusson And Kligman (Maximisation Test) 24879 GLP: Yes Published: No BVL-2309225, ASB2012-11440	Yes	HAG
124	Haferkorn, J.	2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 24604 GLP: Yes Published: No BVL-2309131, ASB2012-11400	Yes	HAG
125	Haferkorn, J.	2010	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) LPT 24607 GLP: Yes Published: No BVL-2309233, ASB2012-11444	Yes	HAG
126	Haferkorn, J.	2010	Acute Inhalation Toxicity Study of Glyphosate TC in Rats LPT 24875 GLP: Yes Published: No BVL-2309153, ASB2012-11410	Yes	HAG
127	Haferkorn, J.	2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 24876 GLP: Yes Published: No BVL-2309129, ASB2012-11399	Yes	HAG
128	Hardell, L., Eriksson, M.	1999	A case-control study of non-Hodgkin lymphoma and exposure to pesticides Cancer vol.85, 6 (1999) 1353-1360 GLP: No Published: Yes BVL-2309788, ASB2012-11838	No	MOD
129	Hardell, L., Eriksson, M., Nordstrom, M.	2002	Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies page 1043-1049	No	LIT

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			Leukemia and Lymphoma, 2002 VoI. 43 5), pp. 1043-1049 GLP: No Published: Yes BVL-2309790, ASB2012-11839		
130	Hatakenaka	1995	HR-001: Teratogenicity Study in Rats IET 94-0152 GLP: Yes Published: No BVL-2309444, ASB2012-11497	Yes	ALS
131	Heath, J.; Strutt, A.; Hudson, P.; Iswariah, V.	1993	Glyphosate: 3 week toxicity study in rats with dermal administration 7839 ! IRI 450881 BVL-1344993, TOX9552367	Yes	BAY CAD CHE DOW MOD MOT NUD
132	Heenehan, P. R.; Braun, W. G.; Rinehart, W. E.; Oleson, F. B.	1978	Acute oral LD50 of Glyphosate in rats 4-5438 ! 4880-77 ! BDN-77-428 BVL-2309107, Z35541	Yes	MON
133	Hideo, U.	1995	HR-001: Primary Eye Irritation study in rabbits IET 95-0034 GLP: Yes Published: No BVL-2309201, ASB2012-11430	Yes	ALS
134	Hideo, U.	1995	HR-001: Primary Dermal irritation study in rabbits IET 95-0035 GLP: Yes Published: No BVL-2309175, ASB2012-11420	Yes	ALS
135	Hideo, U.	1995	HR-001: Dermal sensitisation study in Guinea pigs IET 95-0036 GLP: Yes Published: No BVL-2309227, ASB2012-11441	Yes	ALS
136	Hodge, M. C. E.	1996	First revision to Glyphosate acid: 90 day feeding study in dogs CTL/P/1802 ! PD 0674 BVL-2154312, TOX2000-1991	Yes	SYD SYN
137	Hojo, H.	1995	HR-001: A Teratogenicity Study in Rabbits IET 94-0153 GLP: Yes Published: No BVL-2309446, ASB2012-11498	Yes	ALS
138	Honarvar, N.	2008	Glyphosate Technical - Micronucleus Assay in Bone Marrow Cells of the Mouse 1158500 GLP: Yes Published: No BVL-2309339, ASB2012-11483	Yes	SYN
139	Horner, S.A	1996	Glyphosate acid: Acute neurotoxicity study in rats CTL/P/4866 GLP: Yes Published: No BVL-2309464, ASB2012-11500	Yes	SYN
140	Howe, R. K.; Chott, R. C.; McClanahan, R. H.	1988	The metabolism of glyphosate in Sprague/Dawley rats. Part II. Identification, characterization, and quantitation of Glyphosate and its metabolites after intravenous and oral administration MSL-7206 ! 206300	Yes	BAY CAD CHE DOW MOD MON MOT NUD

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			BVL-1344949, TOX9552357		
141	IARC	2015	Glyphosate. IARC Monographs - 112 ASB2015-8421		
142	Jensen, J. C.	1991	Mutagenicity test: Ames salmonella assay with Glyphosate, batch 206-JaK-25-1 12323 BVL-1345005, TOX9552371	No	BAY CAD CHE DOW MOD MOT NUD
143	Jensen, J. C.	1991	Mutagenicity test: Micronucleus test with Glyphosate, batch 206-JaK-25-1 12324 BVL-1345016, TOX9552374	Yes	BAY CAD CHE DOW EGT MOD MOT NUD
144	Jensen, J. C.	1991	Mutagenicity test: In vitro mammalian cell gene mutation test with Glyphosate, batch 206-JaK-25-1 12325 BVL-1345007, TOX9552372	No	BAY CAD CHE DOW MOD MOT NUD
145	JMPR;	2004	WORLD HEALTH ORGANIZATION and FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, Rome: Pesticide residues in food – 2004; Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues Rome, Italy, 20–29 September 2004 ASB2008-6266		
146	Johnson, D. E.	1982	21-day dermal toxicity study in rabbits IR-81-195 ! 401-168 BVL-1344994, TOX9552366	Yes	BAY CAD CHE DOW MOD MON MOT NUD
147	Johnson, I. R.	1997	Glyphosate acid: Eye irritation to the rabbit CTL/P/5138 ! FB 5378 BVL-2154309, TOX2000-1986	Yes	SYD SYN
148	Kachuri, L.; Demers, P. A.; Blair, A. et al.	2013	Multiple pesticide exposures and the risk of multiple myeloma in Canadian men DOI: 10.1002/ijc.28191 ! page 1846-1858 Int. J. Cancer: 133, 1846–1858 (2013) GLP: No Published: Yes BVL-2716322, ASB2014-8030	Yes	LIT
149	Karunanayake, C.P., Spinelli, J.J., McLaughlin, J.R., Dosman, J.A., Pahwa, P., McDuffie, H.H.	2011	Hodgkin Lymphoma and Pesticides Exposure in Men: A Canadian Case-Control Study Journal of Agromedicine vol.17, 1 (2011) 30-39 GLP: No Published: Yes BVL-2309844, ASB2012-11865	No	LIT
150	Kimmel, G.L.; Kimmel, C.A.; Williams, A.L.; DeSesso, J.M.;	2013	Evaluation of developmental toxicity studies of Glyphosate with attention to cardiovascular development page 79-95 Critical Reviews in Toxicology 2013; 43(2): 79-95. GLP: No Published: Yes BVL-2716230, ASB2013-3462	Yes	LIT
151	Kinoshita, M.	1995	HR-001: 13-week Subchronic Oral Toxicity Study in Rats IET 94-0138 GLP: Yes Published: No	Yes	ALS

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			BVL-2309258, ASB2012-11452		
152	Kitazawa, T.	2013	IET historical control data on milignant lymphoma incidence in control ICR (Crj:CD-1) mice HR-001: Carcinogenicity study in mice (IET 94-0151) 13-C015 Institute of Environmental Toxicology GLP: No Published: No BVL-2716297, ASB2014-9146	No	EGT
153	Knezevich, A. L.; Hogan, G. K.	1983	A chronic feeding study of Glyphosate (Roundup technical) in mice 77-2061 ! (BDN-77-420) BVL-1345024, TOX9552381	Yes	BAY CAD CHE DOW MOD MON MOT NUD
154	Knowles, S. L.; Mookherjee, C. R.	1996	[14C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat 1413/2-1011 GLP: Yes Published: No BVL-2309072, ASB2012-11380	Yes	NUF
155	Koichi, E.	1995	HR-001: Acute inhalation toxicity study in rats IET 94-0155 GLP: Yes Published: No BVL-2309147, ASB2012-11407	Yes	ALS
156	Koller, V. J.; Fürhacker, M.; Nersesyan, A. et al.	2012	Cytotoxic and DNA-damaging properties of Glyphosate and Roundup in human-derived buccal epithelial cells DOI 10.1007/s00204-012-0804-8 Arch Toxicol (2012) 86: 805–813 GLP: No Published: Yes BVL-2716316, ASB2014-7618	Yes	LIT
157	Komura, H.	1995	HR-001: Acute oral toxicity study in mice IET 94-0133 GLP: Yes Published: No BVL-2309088, ASB2012-11383	Yes	ALS
158	Komura, H.	1995	HR-001: Acute oral toxicity study in rats IET 94-0134 GLP: Yes Published: No BVL-2309086, ASB2012-11382	Yes	ALS
159	Komura, Hitoshi	1995	HR-001: Acute dermal toxicity study in rats IET 94-0154 GLP: Yes Published: No BVL-2309123, ASB2012-11396	Yes	ALS
160	Koutros, S.; Andreotti, G.; Berndt, S. I. et al.	2011	Xenobiotic-metabolizing gene variants, pesticide use, and the risk of prostate cancer page 615-623 Pharmacogenetics and Genomics 2011, Vol 21 No 10 GLP: No Published: Yes BVL-2716382, ASB2014-9594	No	LIT
161	Krüger, M.; Schrödl, W.; Pedersen, I; Shehata, A. A.	2014	Detection of Glyphosate in malformed piglets 10.4172/2161-0525.1000230 ! ISSN: 2161-0525 JEAT Environmental & Analytical Toxicology vol.Volume 4, Issue 5 (2014) ASB2014-8935		

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
162	Kuhn, J. O.; Harrison, L. V.	1996	CHA 440: Primary eye irritation study in rabbits 2981-96 ! S9-FF81-4.C41 STILLMEADOW, Inc. BVL-1344970, TOX1999-881	Yes	BAY CAD CHE DOW MOD MOT NUD
163	Kumar, D.P.S.	2001	Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice Toxi: 1559.CARCI-M GLP: Yes Published: No BVL-2309396, ASB2012-11491	Yes	FSG
164	Kuwahara	1995	HR-001: 13-week Oral Subchronic Toxicity Study in Mice IET 94-0136 GLP: Yes Published: No BVL-2309260, ASB2012-11453	Yes	ALS
165	Kyomu, M.	1995	HR-001: In vitro cytogenetics test IET 94-0143 GLP: Yes Published: No BVL-2309317, ASB2012-11475	No	ALS
166	Landgren, O., Kyle, R.A., Hoppin, J.A., Freeman, L.E.B., Cerhan, J.R., Katzmann, J.A., Rajkumar, S.V., Alavanja, M.C.	2009	Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study DOI 10.1182/blood-2009-02-203471 GLP: No Published: Yes BVL-2309874, ASB2012-11875	No	LIT
167	Lankas, G. P.	1981	A lifetime feeding study of Glyphosate in rats - Data evaluation report 77-2062 BVL-2154319, TOX2000-1997		SYD
168	Lankas, G. R.	1981	Lifetime feeding study of Glyphosate (Roundup technical) in rats 77-2062 ! BDN-77-416 BVL-2309378, TOX2000-595		CAD DOW MON MOT
169	Lee, H-L., Chen, K.-W., Chi, C.-H., Huang, J.-J., Tsai, L.-M.	2000	Clinical presentations and prognostic factors of a glyphosate-surfactant herbicide intoxication: a review of 131 cases Academic Emergency Medicine (paper) vol.7, 8 (2000) 906-910 GLP: No Published: Yes BVL-2309492, ASB2012-11512	No	LIT
170	Lee, W.J., Colt, J.S., Heineman, E.F., McComb, R., Weisenburger, D.D., Lijinsky, W., Ward, M.H.	2005	Agricultural pesticide use and risk of glioma in Nebraska, United States Occupational and Environmental Medicine vol.62 (2005) 786-792 GLP: No Published: Yes BVL-2309886, ASB2012-11882	No	LIT
171	Lee, W.J., Lijinsky, W., Heineman, E.F., Markin, R.S., Weisenburger, D.D., Ward, M.H.	2004	Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus Occupational and Environmental Medicine 61 (9):743-749 vol.61, 9 (2004) 743-749 GLP: No Published: Yes	No	LIT

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			BVL-2309888, ASB2012-11883		
172	Leuschner, J.	1995	Metabolism study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley rats 9202/95 BVL-2332809, TOX9650071	Yes	FSG
173	Leuschner, J.	2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC In Rabbits 24877 GLP: Yes Published: No BVL-2309173, ASB2012-11419	Yes	HAG
174	Leuschner, J.	2009	Acute Eye Irritation/Corrosion Test Of Glyphosate TC In Rabbits 24878 GLP: Yes Published: No BVL-2309199, ASB2012-11429	Yes	HAG
175	Leuschner, J.	2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits LPT 23913 GLP: Yes Published: No BVL-2309177, ASB2012-11421	Yes	HAG
176	Leuschner, J.	2009	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits LPT 23914 GLP: Yes Published: No BVL-2309205, ASB2012-11432	Yes	HAG
177	Leuschner, J.	2010	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits LPT 24605 GLP: Yes Published: No BVL-2309179, ASB2012-11422	Yes	HAG
178	Leuschner, J.	2010	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits LPT 24606 GLP: Yes Published: No BVL-2309207, ASB2012-11433	Yes	HAG
179	Levine, S.	2012	EDSP assays and regulatory safety studies provide a weight of evidence that Glyphosate is not an endocrine disruptor page 128 ASB2014-9609		
180	Li, A. P.	1983	CHO/HGPRT gene mutation assay with Glyphosate ML-83-155 ! 830079 BVL-1345008, TOX9552369	No	BAY CAD CHE DOW MOD MON MOT NUD
181	Li, A. P.	1983	In vivo bone marrow cytogenetics study of Glyphosate in Sprague-Dawley rats ML-83-236 ! 830083 BVL-1345015, TOX9552375	Yes	BAY CAD CHE DOW MOD MON MOT NUD

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182	Li, A. P.; Long, T. J.	1988	An evaluation of the genotoxic potential of Glyphosate Page: 537-546 ! L 361 BVL-2146649, TOX9500253	Yes	BCL GTT LIT
183	Lioi, M. B.; Scarfi, M. R.; Santoro, A. et al.	1998	Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro Page: 13-20 Mutation Research 403 1998. 13–20. GLP: No Published: Yes BVL-2716170, ASB2013-9836	No	LIT
184	Lioi, M. B.; Scarfi, M. R.; Santoro, A. et al.	1998	Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed in vitro to Glyphosate, Vinclozolin, Atrazine and DPX-E9636 Page: 39-46 Environmental and Molecular Mutagenesis 32: 39-46 (1998). GLP: No Published: Yes BVL-2716169, ASB2013-9837	No	LIT
185	Lopez, S. L.; Aiassa, D.; Benitez-Leite, S.; Lajmanovich, R.; Manas, F.; Poletta, G.; Sanchez, N.; Simoniello, M. F.; Carrasco, A. E.;	2012	Pesticides used in South American GMO-based agriculture: A review of their effects on humans and animal models doi.org/10.1016/B978-0-444-59389-4.00002-1 ! page 41-75 Advances in Molecular Toxicology Volume 6. GLP: No Published: Yes BVL-2716286, ASB2013-10534	Yes	LIT
186	Macpherson, D.	1996	Glyphosat acid: Biotransformation in the rat CTL/P/5058 GLP: Open (1) Yes (3) Published: No BVL-2154301, TOX2000-1981	Yes	SYD SYN
187	Manas, F.; Peralta, L.; Raviolo, J.; Ovando, H. G.; Weyers, A.; Ugnia, L.; Gonzalez Cid, M.; Larripa, I.; Gorla, N.	2009	Genotoxicity of Glyphosate assessed by the comet assay and cytogenetic tests page 37-41 Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests GLP: No Published: Yes BVL-2309908, ASB2012-11892	No	LIT
188	Mañas, F.; Peralta, L.; Ugnia, L. et al.	2013	Oxidative stress and comet assay in tissues of mice administered Glyphosate and Ampa in drinking water for 14 days page 67-75 Journal of Basic & Applied Genetics GLP: No Published: Yes BVL-2716300, ASB2014-6909	No	LIT
189	McDonald, P.; Anderson, B. T.	1989	Glyphosate technical: Acute inhalation toxicity study in rats (limit test) 5993 ! IRI 642062 BVL-1344964, TOX9552329	Yes	BAY CAD CHE DOW MOD MOT NUD
190	McDuffie, H.H., Pahwa, P., McLaughlin, J.R., Spinelli, J.J., Fincham, S.,	2001	Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross Canada study of pesticides and health CanEpi 10:1155-1163 Cancer Epidemiol Biomarkers Prev vol.10, 11 (2001)	No	LIT

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
	Dosman, J.A., Robson, D., Skinnider, L.F., Ch		1155-1163 GLP: No Published: Yes BVL-2009742, ASB2011-364		
191	McEwen, A. B.	1995	HR-001: Metabolism in the rat SNY 332/951256 GLP: Yes Published: No BVL-2309070, ASB2012-11379	Yes	ALS
192	McQueen, H., Callan, A.C., Hinwood, A.L.	2012	Estimating maternal and prenatal exposure to glyphosate in the community setting. International Journal of Hygiene and Environmental Health (2012) GLP: No Published: Yes BVL-2309926, ASB2012-11898	No	LIT
193	Merkel, D.	2005	Glyphosate Acid Technical: Acute oral toxicity up and down procedure in rats PSL 15274 GLP: Yes Published: No BVL-2309098, ASB2012-11388	Yes	HAG
194	Merkel, D.	2005	Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats - Limit Test PSL 15275 GLP: Yes Published: No BVL-2309133, ASB2012-11401	Yes	HAG
195	Merkel, D.	2005	Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats - Limit Test PSL 15276 GLP: Yes Published: No BVL-2309157, ASB2012-11412	Yes	HAG
196	Merkel, D.	2005	Eye Irritation/Corrosion Effects in rabbits (Oryctolagus cuniculus) of Glyphosate 95 TC PSL 15277 GLP: Yes Published: No BVL-2309211, ASB2012-11435	Yes	HAG
197	Merkel, D.	2005	Glyphosate Acid Technical - Primary Skin Irritation Study in Rabbits PSL 15278 GLP: Yes Published: No BVL-2309183, ASB2012-11424	Yes	HAG
198	Meyer-Carrive, I.; Bolt, A. G.	1994	Acute dermal toxicity of Glyphosate technical in the rat T1586.3.A BVL-2332616TOX9500378	Yes	MAR SIN
199	Milburn, G. M.	1996	Glyphosate acid: One year dietary toxicity study in rats CTL/P/5143 ! PR 1012 BVL-2154318, TOX2000-1998	Yes	SYD SYN
200	Mink, P. J.; Mandel, J. S.; Sceurman, B. K. et al.	2012	Epidemiologic studies of Gyphosate and cancer: A review page 440-452 Regulatory Toxicology and Pharmacology 63 (2012) 440–452 GLP: No Published: Yes BVL-2716296, ASB2014-9617	No	LIT

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
201	Mink, P.J., Mandel, J.S., Lundin, J.I., Sceurman, B.K.	2011	Epidemiologic studies of glyphosate and non-cancer health outcomes: A review Regulatory Toxicology and Pharmacology vol.61, 2 (2011) 172-184 GLP: No Published: Yes BVL-2309938, ASB2012-11904	No	LIT
202	Mladinic, M., Berend, S., Vrdoljak, A.L., Kopjar, N., Radic, B., Zeljezic, D.	2009	Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro Environmental and Molecular Mutagenesis vol.50, 9 (2009) 800-807 GLP: No Published: Yes BVL-2309942, ASB2012-11906	No	LIT
203	Mladinic, M., Perkovic, P., Zeljezic, D.	2009	Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay Toxicol Lett vol.189, 2 (2009) 130-137 GLP: No Published: Yes BVL-2309944, ASB2012-11907	No	LIT
204	Monge, P., Wesseling, C., Guardado, J., Lundberg, I., Ahlbom, A., Cantor, K.P., Weideroass, E., Partanen, T.	2007	Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica Scandinavian Journal of Work Environment & Health vol.33, 4 (2007) 293-303 GLP: No Published: Yes BVL-2309948, ASB2012-11909	No	LIT
205	Monroy, C.; Cortes, A.; Sicard, D. et al.	2005	Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate page 335-345 GLP: No Published: Yes BVL-2309950, ASB2012-11910		LIT
206	Mose, T.; Kjaerstad, M. B.; Mathiesen, L. et al.	2008	Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system page 984-991 GLP: No Published: Yes BVL-2309958, ASB2012-11914		LIT
207	Moxon, M. E.	1996	Glyphosate acid: Developmental toxicity study in the rabbits CTL/P/5009 ! RB 0709 BVL-2154323, TOX2000-2002	Yes	SYD SYN
208	Moxon, M. E.	2000	Glyphosate acid: Multigeneration reproduction toxicity study in rats CTL/P/6332 ! RR 0784 BVL-2154321, TOX2000-2000	Yes	SYD SYN
209	Moxon, M. E.	2002	Glyphosate acid: Developmental toxicity study in the rat - Amendment - 001 CTL/P/4819 ! RR0690 Central Toxicology Laboratory GLP: Yes Published: No BVL-2154322, ASB2012-10080	Yes	EGT SYD SYN Syngenta Agro
210	Multigner, L., Ndong, J.R.,	2008	Environmental pollutants and prostate cancer: epidemiological data	No	LIT

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
	Oliva, A., Blanchet, P.		Gynecol Obstet Fertil vol.36, 9 (2008) 848-856 GLP: No Published: Yes BVL-2309964, ASB2012-11917		
211	Nagy, K.	2011	Glyphosate Technical - Acute inhalation Toxicity Study (Nose-only) in the Rat 11/054-004P GLP: Yes Published: No BVL-2309165, ASB2012-11415	Yes	SYN
212	Nakashima, N.	1997	HR-001: 12-Month Oral Chronic Toxicity Study in Dogs IET 94-0157 GLP: Yes Published: No BVL-2309276, ASB2012-11458	Yes	ALS
213	Ndong, J.R., Blanchet, P., Multigner, L.	2009	Pesticides and prostate cancer: epidemiological data Bulletin Du Cancer vol.96, 2 (2011) 171-180 GLP: No Published: Yes BVL-2309974, ASB2012-11922	No	LIT
214	Nordström, M.; Hardell, L.; Magnuson, A.; Hagberg, H.; Rask-Andersen, A.	1998	Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study Page: 2048-2052 British Journal of Cancer (1998) 77(11), 2048-2052. GLP: No Published: Yes BVL-2716207, TOX1999-687		BVL DOW LIT
215	Pahwa, P. P.; Karunanayak, C. P.; Dosman, J. A. et al.	2011	Soft-tissue sarcoma and pesticides exposure in men results of a canadian case-control study page 1279-1286 JOEM, Volume 53, Number 11, November 2011 GLP: No Published: Yes BVL-2716393, ASB2014-9625	Yes	LIT
216	Pahwa, P., Karunanayake, C.P., Dosman, J.A., Spinelli, J.J., McDuffie, H.H., McLaughlin, J.R.	2011	Multiple Myeloma and Exposure to Pesticides: A Canadian Case-Control Study Journal of Agromedicine vol.17, 1 (2012) 40-50 GLP: No Published: Yes BVL-2309996, ASB2012-11987	No	LIT
217	Parker, R. M.	1993	90 day range finding study of glyphosate in rats TSI 011-0001 BVL-2309252, TOX9650149	Yes	ALK
218	Patel, N. N.	2012	Micronucleus test of Glyphosate TGAI in mice 120709 ! 485-1-06-4696 ! DR-0112-6927-003 ! 10001701-27-1 JAI Research Foundation (JRF) GLP: Yes Published: No BVL-2715972, ASB2014-9277	Yes	DOW
219	Paumgartten, F. J. R.	2012	Pesticide exposure and poor pregnancy outcomes: weaknesses of the evidence // Exposição a agrotóxicos e resultados adversos da gravidez: a fragilidade da evidência Cad. Saúde Pública, Rio de Janeiro, 28(10):2009-2012. GLP: No Published: Yes BVL-2716287, ASB2013-10538	No	LIT

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220	Peluso, M.; Munnia, A.; Bolognesi, C.; Parodi, S.	1997	32P-Postlabeling detection of DNA adducts in mice treated with the herbicide Roundup page 55-59 Environmental and Molecular Mutagenesis 31:55±59 (1998) BVL-2310014, TOX1999-318		BVL DOW LIT
221	Perry, C. J.; Atkinson, C.; Strutt, A.; Henderson, W.; Hudson, P.	1991	Glyphosate: 13 week dietary toxicity study in rats 7136 ! IRI 437876 BVL-1344987, TOX9552364	Yes	BAY CAD CHE DOW MOD MOT NUD
222	Perry, C. J.; Atkinson, C.; Strutt, A.; Hudson, P.; Jones, M.	1991	Glyphosate: 13 week dietary toxicity study in mice 7024 ! IRI 437918 BVL-1344988, TOX9552363	Yes	BAY CAD CHE DOW MOD MOT NUD
223	Pinto, P.J.	1996	Glyphosate acid: 21-day dermal toxicity study in rats CTL/P/4985 GLP: Yes Published: No BVL-2309288, ASB2012-11461	Yes	SYN
224	Pooles, A.	2014	Glyphosate: Acute oral toxicity in the rat - fixed dose method 41401853 GLP: Yes Published: No BVL-2715934, ASB2014-9147	Yes	Albaugh
225	Pore, M. P.; Bhide, M. B.; Naik, P. Y.	1993	Skin sensitisation test in guinea-pigs with Glyphosate technical 95% min of Excel Industries Ltd., Bombay. IIT 1230 TOX9650652	Yes	LUX
226	Powles, P.; Hopkins, R.	1992	(14C)-glyphosate: Absorption and distribution in the rat - preliminary study 6365-676/1 BVL-1344948, TOX9552358	Yes	BAY CAD CHE DOW MOD MOT NUD
227	Powles, P.; Hopkins, R.	1992	(14C)-glyphosate: Absorption, distribution, metabolism and excretion in the rat 7006-676/2 BVL-2005461, TOX9300343	Yes	CHE DOW GTT MOD
228	Prakash, P.J.	1999	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) 1816 / 1817-R.FST GLP: Yes Published: No BVL-2309264, ASB2012-11455	Yes	FSG
229	Rank, J.; Jensen, A. G.; Skov, B. et al.	1992	Genotoxicity testing of the herbicide roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test, and Allium anaphase-telephase test Mutat. Res. (1992) 29-36 GLP: Open Published: Open Z82234	Yes	
230	Rattray, N. J.	1996	Glyphosate acid: 4-hour acute inhalation toxicity study in rats	Yes	SYD SYN

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			CTL/P/4882 ! HR 2284 BVL-2154307, TOX2000-1984		
231	Reagan, E. L.; Laveglia, J.	1988	Acute oral toxicity of Glyphosate Batch/lot/nbr no. XLI-55 in Sprague/Dawley rats 88.2053.007 ! FD-88-29 BVL-2309105, Z35389	Yes	MON
232	Reagan, E. L.; Laveglia, J.	1988	Acute dermal toxicity of Glyphosate Batch/lot/nbr no. XLI-55 in new zealand white rabbits 88.2053.008 ! FD-88-29 BVL-1344960, TOX9552325	Yes	BAY CAD CHE DOW MOD MON MOT NUD
233	Reagan, E. L.; Laveglia, J.	1988	Primary eye irritation study of Glyphosate Batch/lot/nbr no. XLI-55 in new zealand white rabbits 88.2053.009 ! FD-88-29 BVL-2309215, Z35395	Yes	MON
234	Reyna, M. S.	1990	Two generation reproduction feeding study with Glyphosate in sprague-dawley rats + Appendices 1-6 MSL-10387 BVL-1345027, TOX9552387	Yes	BAY CAD CHE DOW MOD MOT NUD
235	Riberri do Val, R.	2007	Bacterial reverse mutation test (Ames Test) for Glifosato Técnico Helm 3393/2007-2.0AM-B GLP: Yes Published: No BVL-2309299, ASB2012-11466	No	HAG
236	Richeux, F.	2006	Glyphosate Technical: Skin Sensitisation in the Guinea Pig - Magnusson and Kligman Maximisation method 2060/009 (SMK-PH-05- GLP: Yes Published: No BVL-2309241, ASB2012-11448	Yes	NUF
237	Ridley, W.P.; Mirly, K.	1988	The metabolism of Glyphosate in Sprague/Dawley rats. I. Excretion and tissue distribution of Glyphosate and its metabolites following intravenous and oral administration MSL-7215 ! EHL 86139 ! ML-86-438 BVL-1344950, TOX9552356	Yes	BAY CAD CHE DOW MOD MON MOT NUD
238	Roe, F. J. C.; Tucker, M. J.;	1974	Recent developments in the design of carcinogenicity tests on laboratory animals Proc. Europ. Soc. Stud. Drug Tox., 15:171-177 (1974) ASB2015-2534		
239	Rossberger, St.	1994	Glyphosat: DNA repair test with primary rat hepatocytes 931564 ! 94-03-28 ro GLP: Open (4) Yes (7) Published: No (6) Open (5) BVL-2327069, TOX9400697		FSG
240	Roth, M.	2012	Glyphosate technical - Micronucleus assay in bone marrow cells of the mouse 1479200 ! TK0112981 Harlan Cytotest Cell Research GmbH (Harlan-CCR) GLP: Yes Published: No BVL-2716029, ASB2014-9333	Yes	Syngenta Agro
241	Schinasi, L.; Leon, M. E.;	2014	Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: A systematic review and meta-analysis		

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			doi:10.3390/ijerph110404449 ASB2014-4819		
242	Schreib, G.	2012	Reverse mutation assay using Bacteria (Salmonella typhimurium) with Glyphosate tech. 126159 BSL Bioservice Scientific Laboratories GmbH GLP: Yes Published: No BVL-2715924, ASB2014-9133	No	INA
243	Schroeder, R. E.; Hogan, G. K.	1981	Three generation reproduction study in rats with Glyphosate 77-2063 ! (BDN 77-417) BVL-1345029, TOX9552385	Yes	BAY CAD CHE DOW MOD MON MOT NUD
244	Séralini, G. E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Spiroux de Vendomois, J.	2012	Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize Page: 4221-4231 Food and Chemical Toxicology 50 (2012) 4221–4231 GLP: No Published: Yes BVL-2716397, ASB2012-15514	No	LIT
245	Sharp, V. M.	1995	Final report for oral and dermal LD 50 tests with Sanachem Glyphosate acid technical in rats, limit test 00917 BVL-2333109, TOX9650909	Yes	DOE SLE
246	Sharp, V. M.	1995	Final report for oral and dermal LD 50 tests with Sanachem Glyphosate 62 % IPA in rats, limit test 00926 BVL-2333108, TOX9650910	Yes	DOE SLE
247	Sher, S. P.	1974	Review article - Tumors in control mice: Literature tabulation Toxicol. Appl. Pharmacol. 30(1974)337-359 GLP: Open Published: Open Z22020	Yes	
248	Shirasu, Y.; Moriya, M.; Ota, T.; Ohta, T.	1978	Glyphosate: The report of mutagenic study with bacteria for CP 67573 - Microbial mutagenicity testing on CP67573 ET-78-241 BVL-1345064, TOX9552368	No	BAY CAD CHE DOW MOD MON MOT NUD
249	Simon, C.	2009	Glyphosate Technical: Acute oral toxicity study in rat C22864 GLP: Yes Published: No BVL-2309090, ASB2012-11384	Yes	EXC
250	Simon, C.	2009	Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs - Maximization-Test C22908 GLP: Yes Published: No BVL-2309229, ASB2012-11442	Yes	EXC
251	Snell, K.	1994	Glyphosate: Acute oral toxicity (limit test) in the rat 710/14 BVL-2332785, TOX9500245	Yes	HPQ

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
252	Snell, K.	1994	Glyphosate: Acute dermal toxicity (limit test) in the rat 710/15 BVL-2332786, TOX9500246	Yes	HPQ
253	Snell, K.	1994	Glyphosate: Magnusson & Kligman maximisation study in the guinea pig 710/19 BVL-2332789, TOX9500250	Yes	HPQ
254	Sokolowski, A.	2007	Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05068) 1061401 GLP: Yes Published: No BVL-2309293, ASB2012-11463	No	NUF
255	Sokolowski, A.	2007	Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05070) 1061402 GLP: Yes Published: No BVL-2309295, ASB2012-11464	No	NUF
256	Sokolowski, A.	2007	Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05067) 1061403 GLP: Yes Published: No BVL-2309297, ASB2012-11465	No	NUF
257	Sokolowski, A.	2009	Glyphosate technical - Salmonella typhimurium and Escherichia coli Reverse Mutation Assay 1264500 GLP: Yes Published: No BVL-2309315, ASB2012-11474	No	SYN
258	Sokolowski, A.	2010	Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine 1332300 GLP: Yes Published: No BVL-2309307, ASB2012-11470	No	HAG
259	Son, W.-C.; Gopinath, C.;	2004	Early occurrence of spontaneous tumors in CD-1 mice and Sprague–Dawley rats DOI: 10.1080/01926230490440871 Toxicologic Pathology, 32:371–374, 2004 ASB2015-2533		
260	Sribanditmongkol, P.; Jutavijittum, P.; Pongraveevongsa, P.; Wunnapuk, K.; Durongkadech, P.	2012	Pathological and toxicological findings in Glyphosate-surfactant herbicide fatality Page: 234-237 Am J Forensic Med Pathol 2012;33: 234Y237 GLP: No Published: Yes BVL-2716398, ASB2014-9731	No	LIT
261	Stout, L. D.; Johnson, C. W.	1987	90 day study of Glyphosate administered in feed to Sprague-Dawley rats MSL-7375 ! ML-86-351 ! EHL 86128 BVL-1344989, TOX9552362	Yes	BAY CAD CHE DOW MOD MON MOT NUD
262	Stout, L. D.; Ruecker, F. A.	1990	Chronic study of Glyphosate administered in feed to albino rats - Appendix 1-6	Yes	BAY CAD CHE DOW

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			MSL 10495 ! ML-87-148 BVL-1345021, TOX9300244		MOD MON MOT NUD
263	Sugimoto, K.	1997	HR-001: 18-Month Oral Oncogenicity Study in Mice IET 940151 GLP: Yes Published: No BVL-2309415, ASB2012-11493	Yes	ALS
264	Suresh, T. P.	1991	Acute oral toxicity study with Glyphosate technical (FSG 03090 H/05 march 90) in Wistar rats ES.874.AOR ! ES-GPT-AOR ! TOXI-874/1990 BVL-2323967, TOX9551088	Yes	FSG
265	Suresh, T. P.	1991	Acute oral toxicity study with Glyphosate technical (FSG 03090 H/05 march 90) in swiss albino mice ES.875.AOM ! ES-GPT-AOM ! TOXI-875/1990 BVL-2324773, TOX9551089	Yes	FSG
266	Suresh, T. P.	1991	Acute dermal toxicity study with Glyphosate technical (FSG 03090 H/05 march 90) in Wistar rats ES.876.ADR ! ES-GPT-ARD ! TOXI-876/1990 BVL-2332810, TOX9551090	Yes	FSG
267	Suresh, T. P.	1991	Glyphosat techn. (FSG 03090 H/05 March 1990): Teratogenicity study in Wistar rats ES.883.TER-R ! TOXI-883/1991 ! ES-GPT-TER-R BVL-2328595, TOX9551105	Yes	FSG
268	Suresh, T. P.	1992	Glyphosat techn. (FSG 03090 H/05 March 1990): 90 day oral toxicity study in wistar rats TOXI-882/1991 ! ES-GPT-90 OR ! ES-882 90 OR BVL-2326328, TOX9551096	Yes	FSG
269	Suresh, T. P.	1996	Combined chronic toxicity and carcinogenicity study with Glyphosate technical in Wistar rats TOXI-886/1996 ! ES-GPT-C.C-R ! TOXI 886.C.C-R BVL-2309343, TOX9651587	Yes	FSG
270	Suresh, T. P. et al.	1991	28-day dietary study in rats on Glyphosate technical ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR BVL-2326272, TOX9551095	Yes	FSG MOD
271	Suresh, T. P. et al.	1992	Glyphosate technical (FSG 03090 H/05, March 1990): Dominant lethal test in wistar rats 888-DLT ! TOXI-888/1992 ! ES-GPT-DLT BVL-2327264, TOX9551102	Yes	FSG
272	Suresh, T. P. et al.	1993	Glyphosate technical (FSG 03090 H/05 March 1990): Teratogenicity study in rabbits 884-TER-RB ! TOXI-884/1992 ! ES-GPT-TER-RB BVL-2309457, TOX9551106	Yes	FSG
273	Suresh, T. P. et al.	1994	28-day dietary study in rats on glyphosate technical - Amendment ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR GLP: Open Published: No Z102035	Yes	
274	Suresh, T. P. et al.	1994	28-day dietary study in rats on glyphosate technical - Second amendment	Yes	

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR GLP: Open Published: No Z102043		
275	Suresh, T. P.; Ponnanna, D.; Asha, M. et al.	1994	Glyphosate technical (FSG 03090 H/05 March 1990): Genetic toxicology - In vivo mammalian bone marrow cytogenetic test 890-MUT-CH.AB ! TOXI-890/1993 ! ES-GPT-MUT-CH.AB BVL-2327261, TOX9400323	Yes	FSG
276	Suresh, T. P.; Rajendran, S.; Shivakumar S.Hosamath et al.	1993	Glyphosate technical (FSG 03090 H/05 March 1990): Two generation reproduction study in wistar rats 885-RP-G2 ! TOXI-885/1993 ! ES-GPT-RP-G2 BVL-2309427, TOX9300009	Yes	FSG
277	Suresh, T.P.	1993	Glyphosate technical (FSG 03090 H/05 March 1990): Mutagenicity-micronucleus test in swiss albino mice 889-MUT.MN ! TOXI-889/1993 ! ES-GPT-MUT-MN BVL-2327258, TOX9551100	Yes	FSG
278	Taddesse-Heath, L.; Chattopadhyay, S. K.; Dillehay, D. L.; et al.;	2000	Lymphomas and high-level expression of murine leukemia viruses in CFW mice J. Virol. 74(2000)15:6832-6837 ASB2015-2535		
279	Takahashi, K.	1997	HR-001: A two-generation reproduction study in rats IET 96-0031 GLP: Yes Published: No BVL-2309425, ASB2012-11495	Yes	ALS
280	Talvioja, K.	2007	GLYPHOSATE TECHNICAL (NUP05068): Acute dermal toxicity study in rats B02283 GLP: Yes Published: No BVL-2309137, ASB2012-11403	Yes	NUF
281	Talvioja, K.	2007	Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) B02294 GLP: Yes Published: No BVL-2309171, ASB2012-11418	Yes	NUF
282	Talvioja, K.	2007	Glyphosate Technical (NUP 05068): Primary Eye Irritation Study In Rabbits B02305 GLP: Yes Published: No BVL-2309197, ASB2012-11428	Yes	NUF
283	Talvioja, K.	2007	Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test B02316 GLP: Yes Published: No BVL-2309223, ASB2012-11439	Yes	NUF
284	Talvioja, K.	2007	GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study in rats BO2272 GLP: Yes Published: No	Yes	NUF

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			BVL-2309103, ASB2012-11390		
285	Tasker, E. J.; Rodwell, D. E.; Jessup, D. C.	1980	Glyphosate: Teratology study in rats 401-054 ! IR-79-016 BVL-1345031, TOX9552392	Yes	BAY CAD CHE DOW MOD MON MOT NUD
286	Tasker, E. J.; Rodwell, D. E.; Jessup, D. C.	1980	Glyphosate: Teratology study in rabbits 401-056 ! IR-79-018 BVL-1345033, TOX9552390	Yes	BAY CAD CHE DOW MOD MON MOT NUD
287	Tavaszi, J.	2011	Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure) 10/218-001P GLP: Yes Published: No BVL-2309113, ASB2012-11392	Yes	SYN
288	Tavaszi, J.	2011	Glyphosate Technical: Acute eye irritation study in rabbits 10/218-005N GLP: Yes Published: No BVL-2309221, ASB2012-11438	Yes	SYN
289	Thompson, P.	2014	Glyphosate: Reverse mutation assay 'Ames test' using Salmonella typhimurium and Escherichia coli 41401854 GLP: Yes Published: No BVL-2715935, ASB2014-9148	Yes	Albaugh
290	Thompson, P.W.	1996	Technical glyphosate: Reverse mutation assay "Ames test" using Salmonella typhimurium and Escherichia coli 434/014 GLP: Yes Published: No BVL-2309311, ASB2012-11472	No	NUF
291	Tornai, A.	1994	Repeated dose 28-day dermal toxicity study with Glyphosate in rabbits GLY-94-410/N ! MÜF 214/94 BVL-2309284, TOX9650151	Yes	ALK MON
292	Tornai, A.; Kovacs, C.; Rozsnyoi, F. et al.	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute inhalation toxicity in rats GHA-94-403/R BVL-2331355, TOX9650144	Yes	ALK
293	Tornai, A.; Rozsnyoi, F. Turczer, K. et al.	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute oral toxicity in rats GHA-94-401/R BVL-2331353, TOX9650142	Yes	ALK
294	Tornai, A.; Rozsnyoi, F. Turczer, K. et al.	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute dermal toxicity in rats GHA-94-402/R BVL-2331354, TOX9650143	Yes	ALK
295	Török-Bathó, M.	2011	Glyphosate technical - Local lymph node assay in the mouse - Final report amendment 2 10/218-037E GLP: Yes Published: No	Yes	SYN

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			BVL-2309247, ASB2012-11450		
296	Tos, E. G.; Maraschin, R.; Orlando, L.	1994	Glyphosate technical: Acute oral toxicity study in mice 940020 ! PRO629 BVL-2331271, TOX9551624	Yes	IPC
297	Tucker, M. J.	1979	The effect of long-term food restriction on tumours in rodents Int. J. Cancer: 23, 803-807 (1979) GLP: Open Published: Open Z83266	Yes	
298	van de Waart, E. J.	1995	Evaluation of the ability of Glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) 141918 BVL-2146653, TOX9651525	No	GTT
299	Vereczkey,L.; Csanyi, E.	1992	18 month carcinogenicity study of Glyphosate in mice 24 151/92 ! 8010 BVL-2331365, TOX9650154	Yes	ALK
300	Walker, D. J.; Jones, J. R.	1992	Glyphosate technical: Acute oral toxicity (limit test) in the rat 134/37 BVL-2331643, TOX9551810	Yes	BCL
301	Walker, D. J.; Jones, J. R.	1992	Glyphosate technical: Acute dermal toxicity (limit test) in the rat 134/38 BVL-2331645, TOX9551813	Yes	BCL
302	Walker, D. J.; Pateman, J. R.; Jones, J. R.	1991	Luxan Glyphosate techn.: Magnusson & Kligman maximisation study in the guinea pig 349/11 BVL-2142260, TOX9551796	Yes	AGC GTT LUX UPL
303	Wallner, B.	2010	Reverse Mutation Assay using Bacteria (Salmonella typhimurium) with Glyphosate TC BSL 101268 GLP: Yes Published: No BVL-2309309, ASB2012-11471	No	HAG HEL
304	Weichenthal, S., Moase, C., Chan, P.	2010	A review of pesticide exposure and cancer incidence in the Agricultural Health Study cohort Environ Health Perspect vol.118, 8 (2010) 1117-1125 GLP: No Published: Yes BVL-2310122, ASB2012-12048	No	LIT
305	Wood, E., Dunster, J., Watson, P. Brooks, P.	2009	Glyphosate Technical: Dietary combined chronic toxicity / carcinogenicity study in the rat SPL2060-0012 GLP: Yes Published: No BVL-2309391, ASB2012-11490	Yes	NUF
306	Wood, E., Dunster, J., Watson, P., Brooks, P.	2009	Glyphosate Technical: Dietary carcinogenicity study in the mouse SPL 2060-0011 GLP: Yes Published: No BVL-2309412, ASB2012-11492	Yes	NUF

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
307	Wood, E.;	2010	Historical Incidence of Malignant lymphoma in CD-1 Mouse ASB2015-2531		
308	Wrenn, J. M.; Rodwell, D. E.; Jessup, D. C.	1980	Dominant lethal mutagenicity assay with technical Glyphosate in mice 401-064 ! IR-79-014 BVL-1345017, TOX9552377	Yes	BAY CAD CHE DOW MOD MON MOT NUD
309	Wright, N.P.	1996	Technical glyphosate: Chromosome aberration test in CHL cells in vitro 434/015 GLP: Yes Published: No BVL-2309319, ASB2012-11476	No	NUF
310	Yang, W.; Carmichael, S. L.; Roberts, E. M. et al.	2013	Residential agricultural pesticide exposures and risk of neural tube defects and orofacial clefts among offspring in the San Joaquin Valley of California page 1-9 American Journal of Epidemiology Advance Access published February 18, 2014 GLP: No Published: Yes BVL-2716461, ASB2014-9644	No	LIT
311	Yoshida, A.	1996	HR-001: 13-week Oral Subchronic Toxicity Study in Dogs IET 94-0158 GLP: Yes Published: No BVL-2309269, ASB2012-11456	Yes	ALS
312	You, J.	2009	Glyphosate: Acute oral toxicity study (UDP) in rats 12170-08 GLP: Yes Published: No BVL-2309084, ASB2012-11381	Yes	HAG
313	You, J.	2009	Glyphosate - Acute Dermal Toxicity Study in Rats 12171-08 GLP: Yes Published: No BVL-2309121, ASB2012-11395	Yes	HAG
314	You, J.	2009	Glyphosate - Acute Eye Irritation Study in Rabbits 12172-08 GLP: Yes Published: No BVL-2309209, ASB2012-11434	Yes	HAG
315	You, J.	2009	Glyphosate - Acute Dermal Irritation Study in Rabbits 12173-08 GLP: Yes Published: No BVL-2309181, ASB2012-11423	Yes	HAG
316	Zelenak	2011	Glyphosate Technical - Acute Dermal Toxicity Study in Rats - Final Report Amendmend 1 10/218-002P GLP: Yes Published: No BVL-2309143, ASB2012-11405	Yes	SYN
317	Zelenák, V.	2011	Glyphosate Technical - Primary skin irritation study in rabbits - Final report Amendment 1 10/218-006N GLP: Yes Published: No	Yes	SYN

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			BVL-2309195, ASB2012-11427		
318	Zoriki Hosomi, R.	2007	Mammalian Erythrocyte Micronucleus Test for Glifosato Técnico Helm 3393/2007-3.0MN-B GLP: Yes Published: No BVL-2309331, ASB2012-11480	Yes	HAG
319	Zouaoui, K.; Dulaurent, S.; Gaulier, J. M. et al.	2012	Determination of Glyphosate and AMPA in blood and urine from humans: About 13 cases of acute intoxication page e1-e6 Forensic Science International xxx (2012) xxx–xxx GLP: No Published: Yes BVL-2716400, ASB2014-9734	Yes	LIT
320	Alavanja, M.C.R.; Sandler, D.P.; McMaster, S.B. et al.	1996	The agricultural health study page 362-369 Environmental Health Perspectives, Vol. 104, No 4 Published: Yes ASB2015-7849		
321	Blair, A.; Thomas, K.; Coble, J. et al.	2011	Impact of pesticide exposure misclassification on estimates of relative risks in the agricultural health study page 537-541 Occup. Environ. Med. 68(7) doi:10.1136/oem.2010.059469 Published: Yes ASB2015-7868		
322	Dennis, L.K.; Lynch, C.F.; Sandler, D.P. et al.	2010	Pesticide use and cutaneous melanoma in pesticide applicators in the Agricultural Health Study page 812-817 Environmental Health Perspectives, Vol. 118, No 6 doi:10.1289/ehp.0901518 ! PMID:20164001 Published: Yes ASB2015-8439		
323	De Roos, A.J.; Svec, M.A.; Blair, A. et al.	2005	Glyphosate results revisited: De Roos et al. respond page A366-A367 Environmental Health Perspectives, Vol. 113, No 6 doi:10.1289/ehp.113-a366 Published: Yes ASB2015-8437		
324	Lee, W.J.; Sandler, D.P.; Blair, A. et al.	2007	Pesticide use and colorectal cancer risk in the Agricultural Health Study page 339-346 Int. J. Cancer. 121(2) doi:10.1002/ijc.22635 Published: Yes ASB2015-8228		
325	Sorahan, T.	2015	Multiple myeloma and Glyphosate use: A re-analysis of US Agricultural Health Study (AHS) data page 1548-1559 Int. J. Environ. Res. Public Health, Vol. 12 doi:10.3390/ijerph120201548 ASB2015-2284		
326	Brown, L.M.;	1990	Pesticide exposures and other agricultural risk factors for		

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
	Blair, A.; Gibson, R. et al.		leukemia among men in Iowa and Minnesota Page 6585-6591 Cancer Res. 50(20) PMID: 2208120 Published: Yes TOX2003-999		
327	Brown, L. M.; Burmeister, L. F.; Everett, G. D. et al.	1993	Pesticide exposures and multiple myeloma in Iowa men Page 153-156 Cancer Causes and Control, Vol. 4 Published: Yes BVL-1968123, TOX2002-1000		
328	Cantor, K.P.; Blair, A.; Everett, G. et al.	1992	Pesticides and Other Agricultural Risk Factors for Non-Hodgkin's Lymphoma among Men in Iowa and Minnesota Page 2447-2455 Cancer Research, Vol. 52 Published: Yes ASB2015-7885		
329	Lee, W.J.; Cantor, K.P.; Berzofsky, J.A. et al.	2004	Non-Hodgkin's lymphoma among asthmatics exposed to pesticides page 298-302 Int. J. Cancer, Vol. 111 doi 10.1002/ijc.20273 Published: Yes ASB2015-8238		
330	Orsi, L., Delabre, L., Monnereau, A., et al.	2009	Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study page 291-298 Occup. Environ. Med., Vol. 66 doi:10.1136/oem.2008.040972 Published: Yes BVL-2309992, ASB2012-11985		
331	Waddell, B.L.; Zahm, S.H.; Baris, D. et al.	2001	Agricultural use of organophosphate pesticides and the risk of non-Hodgkin’s lymphoma among male farmers (United States) page 509-517 Cancer Causes & Control, Vol. 12, No 6 doi:10.1023/A:1011293208949 PMID:11519759 Published: Yes ASB2015-8037		
332	Hoar Zahm, S.; Weisenburger, D. D.; Babbitt, P. A. et al.	1990	A case control study of non-Hodgkin’s lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in Eastern Nebraska Page 349-356 Epidemiology, Vol. 1, No 5 Published: Yes ASB2013-11501		
333	Ruder, A.M.; Waters, M.A.; Butler, M.A. et al.	2010	Gliomas and farm pesticide exposure in men: The upper midwest health study page 650-657 Archives of Environmental Health, Vol. 59, No 12 doi: 10.1080/00039890409602949		

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Published: Yes ASB2015-8078		
334	JMPR	2016	Joint FAO/WHO Meeting on Pesticide Residues, Geneva, 9–13 May 2016, Summary Report pages: 6 http://www.who.int/foodsafety/jmprsummary2016.pdf?ua=1 Published: Yes ASB2016-4292		
335	EFSA	2015	Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015;13(11):4302 Published: Yes ASB2015-11412		
336	Burgener, A.	1990	Hydrolyses determination of 14C-glyphosate (PMG) at different pH values RCC238500 GLP: Yes, published: No BVL-2442046	No	MON
337	Van Dijk, A.	1992	Photodegradation study of 14C-Glyphosate in water at pH 5,7 and 9 RCC250751 GLP: Yes, published: No BVL-2252558	No	MON
338	Wüthrich, V.	1990	Glyphosate technical: Inherent biodegradability, “Modified Zahn-Wellens test” RCC271653 GLP: Yes, published: No BVL-1934369	No	MON
339	Carrick, T.R.	1991	A study to evaluate ready biodegradability of Glyphosate technical FH-OECD-09RB GLP: Yes, published: No BVL-2325628	No	MON
340	Feil, J.	2009	Ready biodegradability of glyphosate in a monometric respirometry test Report No. 53981163 GLP: Yes, published: No	Yes	NUF
341	Kent, S.J.,Caunter, J.E., Morris, D.S., Johnson, P.A.	1995	Glyphosate acid: Acute toxicity to Bluegill Sunfish (Lepomis macrochirus) BL5553/B SYN GLP: Yes, published: No BVL-2310926	Yes	SYN
342	Dias Correa Tavares, C.M.	2000	Chronic Toxicity of Glifosate Técnico Nufarm to Zebrafish larvae (Brachydanio rerio) RF-D62.16/99 NUF GLP: Yes, published: No BVL-2310938	Yes	NUF
343	Wüthrich, V.	1990	48-Hour Acute toxicity of Glyphosate techn. to Daphnia magna (OECD-Immobilization Test) 272968 CHE	No	CHE

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP: Y, published: N BVL-2310947		
344	Magor, S.E., Shillabeer, N.	1999	Glyphosate acid: Chronic toxicity to Daphnia magna BL6535/B SYN GLP: Yes, published: No BVL-2310962	No	SYN
345	Smyth, D.V., Shillabeer, N., Morris, D.S., Wallace, S.J.	1996	Glyphosate acid: Toxicity to blue-green alga Anabaena flos-aquae BL5698/B SYN GLP: Yes, published: No BVL-2310970	No	SYN
346	Smyth, D.V., Kent, S.J., Morris, D.S., Shearing, J.M., Shillabeer, N.	1996	Glyphosate acid: Toxicity to the marine alga Skeletonema costatum BL5684/B SYN GLP: Yes, published: No BVL-2310972	No	SYN
347	Smyth, D.V., Kent, S.J., Morris, D.S., Cornish, S.K., Shillabeer, N	1996	GLYPHOSATE ACID: Toxicity to duckweed (Lemna gibba) BL5662/B SYN GLP: Yes, published: No BVL-2310988	No	SYN

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toxicology: a practical approach. 2nd ed. Boca Raton: CRC Press, 329–424.

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713–33.

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Phiny, C. and L. Kaensombath (2006). Effect on feed intake and growth of depriving rabbits access to caecotrophes. *Livestock Research for Rural Development*, 18 (3)

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8 ANNEXES

- Final Addendum to the Renewal Assessment Report on Glyphosate (containing the public version of the RAR on glyphosate, Addendum 1 to RAR on glyphosate ("Assessment of IARC Monographs Volume 112 (2015): Glyphosate") and Addendum 1 to RAR on glyphosate, Part Ecotoxicology ("Assessment of IARC Monographs Volume 112 (2015): Glyphosate)
- EFSA Conclusion on pesticide peer review, EFSA Journal 2015;13(11):4302
- Confidential Annex