

Renewal Assessment Report

18 December 2013

Glyphosate

Volume 3

Annex B.6.1

Toxicology and
metabolism

RMS: Germany

Co-RMS: Slovakia

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Contents

B Rapporteur Member State summary, evaluation and assessment of the data and information

B.6	Toxicology and metabolism	1
B.6.1	Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)	4
B.6.1.1	Toxicokinetic and metabolism studies in rats that were not previously evaluated in the EU	4
B.6.1.2	Re-evaluation of previously known studies (mentioned in the 1998 DAR, ASB2010-10302) by the RMS	40
B.6.1.3	Published information	42
B.6.1.4	Data obtained with formulations	44
B.6.2	Acute toxicity including irritancy and skin sensitisation (Annex IIA 5.2)	45
B.6.2.1	Acute oral toxicity	45
B.6.2.2	Acute percutaneous toxicity	80
B.6.2.3	Acute inhalation toxicity	106
B.6.2.4	Skin irritation	142
B.6.2.5	Eye irritation	168
B.6.2.6	Skin sensitisation	201
B.6.3	Short-term toxicity (Annex IIA 5.3)	232
B.6.3.1	Subacute studies (all routes)	232
B.6.3.1.1	Subacute oral studies in rats and dogs	232
B.6.3.1.2	Subacute inhalation studies (rat)	233
B.6.3.1.3	Subacute percutaneous toxicity studies (rabbits and rats)	233
B.6.3.2	Subchronic toxicity (oral)	238
B.6.3.2.1	90-day studies in rats	238
B.6.3.2.2	90-day studies in mice	256
B.6.3.2.3	Oral 90-day and 1-yr studies in dogs	262
B.6.4	Genotoxicity (Annex IIA 5.4)	301
B.6.4.1	<i>In vitro</i> genotoxicity testing – Bacterial assays for gene mutation	301
B.6.4.2	<i>In vitro</i> genotoxicity testing – Tests for gene mutation in mammalian cells	333
B.6.4.3	Tests for DNA damage and repair in mammalian cells and bacteria	337
B.6.4.4	<i>In vitro</i> genotoxicity testing – Tests for clastogenicity in mammalian cells	340
B.6.4.5	<i>In vivo</i> genotoxicity testing (somatic cells) – Metaphase analysis in rodent bone marrow, or micronucleus test in rodents	353
B.6.4.6	<i>In vivo</i> studies in germ cells	371
B.6.4.7	Genotoxicity of formulations (taking into account published data that were released before 2000)	372
B.6.4.8	Published data (released since 2000)	391
B.6.4.8.1	Introduction	391
B.6.4.8.2	General review and analysis considerations	391
B.6.4.8.3	Structure Activity Analysis	398
B.6.4.8.4	Gene Mutation	398
B.6.4.8.5	Chromosome effects	399
B.6.4.8.6	DNA damage and other end points	406
B.6.4.8.7	Human and environmental studies	409
B.6.4.8.8	DNA-Reactivity and carcinogenesis	413
B.6.4.8.9	AMPA and POEA	414

B.6.4.8.10	Genotoxicity Weight of Evidence	416
B.6.5	Long-term toxicity and carcinogenicity (Annex IIA 5.5)	435
B.6.5.1	Long-term toxicity and carcinogenicity in the rat	435
B.6.5.1.1	New studies in rats	435
B.6.5.1.2	Previously known long-term studies in rats (already subject to EU evaluation)	467
B.6.5.2	Carcinogenicity in the mouse	493
B.6.5.3	Published data on carcinogenicity (released since 2000)	518
B.6.6	Reproductive toxicity (Annex IIA 5.6)	538
B.6.6.1	Two generation reproductive toxicity in the rat	539
B.6.6.2	Separate male and female studies	596
B.6.6.3	Three segment designs	596
B.6.6.4	Dominant lethal assay for male fertility	597
B.6.6.5	Cross-matings of treated males with untreated females and vice versa	597
B.6.6.6	Effects on spermatogenesis	597
B.6.6.7	Effects on oogenesis	597
B.6.6.8	Sperm motility and morphology	597
B.6.6.9	Investigation of hormonal activity	597
B.6.6.10	Teratogenicity test by the oral route in the rat	597
B.6.6.11	Teratogenicity test by the oral route in the rabbit	607
B.6.6.12	Published data (released since 2000)	656
B.6.7	Delayed neurotoxicity (Annex IIA 5.7.2)	690
B.6.7.1	Published data on neurotoxicity	702
B.6.8	Further toxicological studies	708
B.6.8.1	Toxicity of the metabolite AMPA	709
B.6.8.2	Mechanistic studies on certain aspects of the toxicity of glyphosate	729
B.6.8.2.1	Studies on mechanism of salivary gland findings	729
B.6.8.1.1	Further studies	741
B.6.8.3	Studies in farm animals	752
B.6.8.3.1	Acute toxicity in goats	753
B.6.8.3.2	Subacute toxicity in cows	766
B.6.8.3.3	Published data	778
B.6.8.4	Further published data (released since 2000)	782
B.6.9	Medical data and information (Annex IIA 5.9)	794
B.6.9.1	Report on medical surveillance on manufacturing plant personnel	794
B.6.9.2	Reports on clinical cases and poisoning incidents	795
B.6.9.3	Observations on exposure of the general population and epidemiological studies	795
B.6.9.4	Clinical signs and symptoms of poisoning and details of clinical tests	796
B.6.9.5	First aid measures	802
B.6.9.6	Therapeutic regimes	803
B.6.9.7	Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion	806
B.6.9.8	Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment	807
B.6.10	Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.11)	808
B.6.11	Acute toxicity including irritancy and skin sensitisation of preparations (Annex IIIA 7.1)	809
B.6.11.1	Summary	809

B.6.11.2	Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for MON 52276.....	810
B.6.11.3	Acute oral toxicity	812
B.6.11.4	Acute percutaneous toxicity	813
B.6.11.5	Acute inhalation toxicity	814
B.6.11.6	Skin irritation.....	815
B.6.11.7	Eye irritation.....	817
B.6.11.8	Skin sensitisation.....	819
B.6.11.9	Supplementary studies for combinations of plant protection products.....	820
B.6.12	Dermal absorption (Annex IIIA 7.6).....	820
B.6.12.1	Dermal absorption of glyphosate from MON 52276 <i>in vitro</i> (Human epidermis).....	821
B.6.12.2	Dermal absorption of glyphosate active substance <i>in vitro</i> (abraded rabbit skin).....	827
B.6.12.3	Publications on dermal absorption of glyphosate	833
B.6.13	Toxicological data on non active substances (Annex IIIA 7.9 and point 4 of the introduction)	834
B.6.13.1	Material safety data sheet for each formulant	834
B.6.13.2	Available toxicological data for each formulant	834
B.6.13.3	Further toxicological data for other potential co-formulants	835
B.6.13.3.1	Toxicological evaluation of the [REDACTED] surfactant (CAS no. 61791-26-2).....	835
B.6.14	Exposure data (Annex IIIA 7.3 to 7.5).....	851
B.6.14.1	Operator exposure (IIIA 7.3.1 and 7.3.2).....	852
B.6.14.1.1	Estimation of operator exposure	852
B.6.14.1.2	Measurement of operator exposure (mixer/loader/applicator) (IIIA 7.3.3)	854
B.6.14.2	Bystander and resident exposure (IIIA 7.4)	855
B.6.14.2.1	Estimation of bystander and resident exposure.....	855
B.6.14.2.2	Measurement of bystander or resident exposure.....	857
B.6.14.3	Worker exposure	857
B.6.14.3.1	Estimation of worker exposure.....	857
B.6.14.3.2	Measurement of worker exposure	858
Appendix 1	Exposure calculations.....	859
A 1.1	Operator exposure calculations (IIIA 7.3).....	859
A 1.2	Bystander and resident exposure calculations (IIIA 7.4).....	868
A 1.3	Worker exposure calculations (IIIA 7.5).....	873
B.6.15	References relied on	874

B.6 Toxicology and metabolism

General introduction and explanation of the approach taken by RMS

This health evaluation of glyphosate is based on the following sources:

- *Toxicological and ADME studies that were submitted by the GTF for this re-evaluation.*
- *Toxicological studies and ADME studies that had been reported in the previous DAR (1998, ASB2010-10302) already and, thus, were part of previous EU evaluation. However, they were subject to re-assessment by the RMS according to current quality standards and were used only when regarded as acceptable or at least supplementary. In very few cases, NOAELs/LOAELs were revised. Unacceptable (old or new) studies were usually deleted with justifications given in the respective sections of Volume 3. In exceptional cases, such studies are still mentioned, i.e., if they were formerly taken into consideration for, e.g., ADI setting.*
- *Scientific publications and other relevant information that were submitted either by the GTF or by third parties or of which the RMS was aware before. It must be emphasised that a large part of the publications was on formulations different from the representative one and, thus, is of limited value for the toxicological evaluation of the active ingredient. With rather few exceptions in the areas of genotoxicity and human data, mainly scientific literature published since 2000 was assessed.*

Due to the large number of submitted toxicological studies, the RMS was not able to report the original studies in detail and an alternative approach was taken instead. The study descriptions and assessments as provided by GTF were amended by deletion of redundant parts (such as the so-called "executive summaries") and new enumeration of tables. Obvious errors were corrected. Each new study was commented by the RMS. These remarks are clearly distinguished from the original submission by a caption, are always written in italics and may be found on the bottom of the individual study summaries.

Furthermore, in Volume 3, assessment was performed on the individual study level. Overall evaluation of the diverse toxicological endpoints was transferred into Volume 1 (section 2.6).

The technical databases that have been used for the literature search include: Web of ScienceSM, BIOSIS Previews®, CAB Abstracts® (CABI), MEDLINE®, and CA Plus (Chemical Abstracts Plus). The searches were made on glyphosate acid, glyphosate salts (including isopropyl amine, potassium, ammonium, and methylamine), and AMPA, and their related chemical names and CAS numbers. Searches based on these search terms were also found suitable to identify publications that consider glyphosate and surfactants (such as polyoxyethylenealkylamines, or POEA) in the context of glyphosate formulations.

Additional publications cited in a recent document prepared by the NGO "Earth Open Source" (Antoniou M, et al., 2011, ASB2011-7202) have also been included in the literature review.

The peer-reviewed publications identified for inclusion during the literature search were reviewed and classified into one of the categories listed below.

- **Category 0 publications:** These are publications in which glyphosate is only mentioned as an example substance or is discussed/studied in a context that is not relevant or related to any of the regulatory sections or the exposure/hazard assessments within this submission; the publication is therefore outside of the scope of this submission.
- **Category 1 publications:** These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and the conclusions fall within the conclusions of the exposure/hazard assessment. The publication is submitted with minimal or no comment or discussion.
- **Category 2 publications:** These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and have conclusions that call into question the endpoints/conclusions in the exposure/hazard assessment. Additionally, Category 2 also includes publications with conclusions that support the risk/hazard assessment, and may be included in discussion of other relevant publications. For selected Category 2 publications, an OECD Tier-II type summary is provided in addition to a reliability assessment (Klimisch rating, see Klimisch et al. 1997, ASB2010-14388); limited comments and critical remarks are provided, as appropriate.
- **Category 3 publications:** These are publications that discuss glyphosate in a context relevant or related to (1) non-regulatory endpoints that need to be addressed as per new Regulation (EC) 1107/2009; or (2) in a context relevant to sensitive allegations that have emerged or could emerge in the media; or (3) in a context relevant to the regulatory dossier sections and have conclusions that are in disagreement with endpoints/conclusions in the exposure/hazard assessment (although the experimental design seems relevant at first glance). An OECD Tier-II type summary is provided and a Klimisch rating assigned, and supplemented with critical review and discussion.
- **Category 'E' publications:** These are peer-reviewed publications that were cited in the Earth Open Source document. This category includes publications that were already captured by the literature search and are addressed within the appropriate discipline, as well as publications that were out of scope of the search (primarily as a result of being published prior to 2001). Publications already captured in the literature search were assigned a Category 1, 2 or 3 rating (as appropriate) in addition to a Category 'E' rating. An OECD Tier-II type summary has been prepared and a Klimisch rating assigned for each of the Category E publications. All Category 'E' publications are reviewed within the appropriate discipline, with most of the reviews provided within the toxicology dossier under Section IIA 5.10.

A full description of the literature search methodology was provided by the GTF in a separate document (Carr and Bleeke, 2012, ASB2012-11583).

Five separate publication subject areas are addressed in the literature review.

- 1. Developmental and Reproductive Toxicity (DART) and Endocrine Disruption (ED)*
- 2. Neurotoxicity*
- 3. Carcinogenicity*
- 4. Genotoxicity*
- 5. Category E and other publications*

The publications on subject areas 1-4 are presented in the chapters on Genotoxicity, Long term toxicity and carcinogenicity, Reproductive Toxicity and Neurotoxicity of the report.

Furthermore, publications are presented in the chapters “Further toxicological studies” and “Medical data”.

Important publications are presented in summaries as quoted from the articles followed by Klimisch ratings and by RMS comments on the paper.

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

Introduction into this chapter by the RMS

In this section, only studies on toxicokinetics and metabolism of glyphosate are reported in detail and commented by the RMS that were not available when the previous evaluation by the EU in the 1990ies was performed. In addition, re-evaluation of those studies which were mentioned in the original monograph (DAR, 1998, ASB2010-10302) has been performed by RMS, mainly with regard to their quality and reliability. For more detailed description of the individual studies from this group, we refer to the old DAR. The outcome of this re-evaluation is briefly given at the end of this section, as well as a short summary of published information. Overall evaluation is presented in Volume 1, including two tables in which the (new and previously known) critical studies and the distribution of glyphosate in excreta and tissues are summarised.

B.6.1.1 Toxicokinetic and metabolism studies in rats that were not previously evaluated in the EU

1st study [REDACTED], 1995)

Reference: IIA, 5.1.1/01

Report: [REDACTED] (1995) HR-001: Metabolism in the rat

Data owner: Arysta Lifescience SAS

Report No.: SNY 332/951256

Date: 1995-08-16, not published, ASB2012-11379

Guidelines: Japanese MAFF, 59 NohSan, Notification No. 4200 (1985)

OECD 417

US-EPA FIFRA 85-1

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Materials and methods

Non-labelled test material: HR-001

Identification: N-(phosphonomethyl)glycine (CAS No. 1071-83-6)

Description: Solid

Lot/Batch #: 061221

Purity: 98.9 %

Stability of test compound: Expiry date 1996-12-20

Radiolabelled test material: [¹⁴C] - HR001

Identification: [¹⁴C] - N-(phosphonomethyl)glycine (CAS No. 1071-83-6)

Position of radiolabel: N-(phosphono[¹⁴C]methyl)glycine

Lot/Batch #: Not reported

Purity: > 98 % (TLC, followed by radioscanning)

Specific activity: 327.7 µCi/mg, 56 mCi/mmol

Stability of test compound:	Not reported
Reference substances:	
Identification:	Aminomethylphosphonic acid (AMPA) (CAS No. 1066-51-9)
Description:	Solid
Lot/Batch #:	09203L2
Purity:	99 %
Stability of test compound:	Not reported
Radiolabelled reference substance:	
Identification:	[¹⁴ C] -Aminomethylphosphonic acid (¹⁴ C-AMPA)
Position of radiolabel:	Amino[¹⁴ C]methyl
Lot/Batch #:	No
Purity:	97.4 %
Specific activity:	2.0 GBq/mmol, 54 mCi/mmol
Stability of test compound:	Not reported
Vehicle and/or positive control:	Water, solubility was increased by addition of sodium hydrogen carbonate
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	
Age:	6-8 weeks (males), 7-9 weeks (females)
Sex:	Males and females
Weight at dosing:	approximately 200 g
Acclimation period:	At least 5 days
Diet/Food:	Standard Laboratory Diet LAD 1 (Special Diet Services, Witham, Essex, UK), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
	During acclimatisation:
	Individual housing in suspended, wire bottom, stainless steel cages.
Housing:	After dosing:
	Excretion-balance experiments - individually in glass metabolism cages
	Blood/plasma kinetics - in stainless steel battery cages
	Tissue distribution – in stainless steel battery cages
Environmental conditions:	Temperature: 21 ± 2 °C
	Humidity: 40 - 60 %
	Air changes: not reported
	12-hour light/dark cycle

Study design and methods

In life dates: not reported

Animal assignment and treatment: Preliminary excretion studies

In two independent experiments two rats (1 male, 1 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 0-6, 6-24, and every 24 h for 7 days in receivers cooled with solid CO₂. Faeces were collected every 24 h for 7 days. Expired air was passed through traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed every 24 h for 7 days after dosing. The interior of the cages were washed with water at sacrifice after 7 days. Samples were analysed immediately or were stored at -20 °C until taken for analysis.

Animal assignment and treatment: Excretion studies

In two independent experiments 10 rats (5 male, 5 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study. Blood was drawn by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following tissues/organs were taken or sampled for radioactivity measurement:

Adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.

Animal assignment and treatment: Plasma concentrations

In two independent experiments 18 rats (9 male, 9 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage. The animals were divided into three groups of six (3 per sex) and blood samples (0.5 mL) were taken from the tail vein into heparinised tubes at the following times from each group.

Group 1: prior to administration, 1, 4, 24 and 96 h

Group 2: 0.25, 2, 6, 48 and 120 h

Group 3: 0.5, 3, 12, 72 and 168 h

Each group was sacrificed upon completion of the specified sampling schedule.

Animal assignment and treatment: Quantitative tissue distribution

In two independent experiments 12 rats (6 male, 6 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage. The animals were divided into two groups of six (3 per sex) and sacrificed by cervical dislocation 6 and 18 h (low dose) or 3 and 9 h (high dose) after dosing, depending on the peak plasma concentrations and half the plasma concentration derived in the blood/plasma kinetics experiments. Data for 168 h (7 days) was provided by the excretion studies. Blood samples were taken by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following tissues/organs were taken or sampled for radioactivity measurement: adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.

Measurement of radioactivity

Faeces were initially extracted by homogenisation with chloroform : 1N HCl (1:1, v/v) followed by further extracts with 1N HCl. After centrifugation radioactivity was measured in both extracts and residues. Samples of urine, plasma, solvent extracts contents of expired air traps, cage washings and other liquid samples were mixed with Special Scintillator MI-31 (Packard Instrument Co. Ltd, Reading, UK) for measurement of radioactivity by liquid scintillation counting (LSC). Samples of faecal residues, gastro-intestinal tract, liver, spleen and whole blood were combusted, absorbed, mixed with scintillation cocktail and analyzed thereafter. Carcasses were digested for 48 h at 55 °C in a solution of 2M NaOH in 30 % Methanol containing Triton X-405 (10 % v/v). Tissue samples suitable for solubilisation were incubated at around 50 °C for 18-24 h with solubiliser and mixed with scintillation cocktail and analyzed thereafter. Radioactivity with less than twice background counts was considered to be below the limit of accurate quantification when performing LSC.

Isolation of the major urinary and faecal metabolites

Samples of urine and faecal extracts from male and female rats were pooled and analysed directly by TLC or HPLC. Radiolabelled metabolite products formed in the rat were identified by co-chromatographic comparison using different systems with the reference compound aminomethyl phosphonic acid (AMPA) or ¹⁴C-AMPA.

Thin layer chromatography (TLC)

TLC was carried out on pre-layered Merck cellulose F plates (0.2 mm, BDH Chemicals Ltd., Poole, UK) using the following development systems:

System 1: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid
(55 : 35 : 5 : 3.5 : 2, v/v/v/w/v)

System 2: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid
(65 : 35 : 2.5 : 3.5 : 2, v/v/v/w/v)

System 5: Methanol : water : acetic acid (67 : 33 : 1, v/v/v)

Radioactivity was detected with a Berthold Linear Analyser controlled by a computer system (Berthold Instruments (UK)) and proportions of radioactive components were measured by integrating the areas under the peaks on the radio chromatogram following subtraction of background levels. Alternatively, components were detected and quantified using a Fuji BAS 2000 Bioimage Analyser. The produced images of radioactive TLC plates were processed to generate quantitative data.

High performance liquid chromatography (HPLC)

Two HPLC methods were used. HPLC system 1 (gradient elution method; column: Spherisorp SAX HPLC column (Hichrom, UK) and guard column, eluent A: water, eluent B: 0.75 M KH₂PO₄, pH 3.35) and HPLC system 2 (isocratic method; column: glyphosate analytical column (BioRad, USA), eluent: 0.005 M KH₂PO₄ + 4 % methanol v/v, pH 2.1) were both linked to an UV- and a radio-detector. A Compaq Prolinea computer with Labchrom software was used to collect and process data from the UV and radio detectors. Samples were co-injected with a mixture of the reference standards. Fractions were collected and radio assayed by LSC.

Results and discussion

Excretion and retention of radioactivity

A preliminary study on two rats per dose (male/female) indicated that more than 90 % of the administered radioactivity was excreted within 7 days by both the low and the high dose

group after a single application of the test substance. Almost no radioactivity could be detected in expired air (about 0.15 %).

The main study with 10 rats per dose confirmed the initial observation (see Table B.6.1-1). During the 7 days observation period 23 % and 19 % (male/female) were excreted in the urine of the low dose group. Slightly higher percentages, 30 % and 29 % (male/female), of total administered radioactivity were detected in urine of the high dose group. The main portion of the radioactivity was detected at both dose levels within the first 48 h in males and females (21 % and 18 %, 10 mg/kg bw; 28 % and 27 %, 600 mg/kg bw). In both dose groups about 75 % of the administered radioactivity could be detected in the faeces of males and females within 7 days (75 % and 84 %, 10 mg/kg bw; 75 % and 74 %, 600 mg/kg bw). Again most of the radioactivity was detected within 48 h after dosing (72 % and 82 %, 10 mg/kg bw; 72 % and 69 %, 600 mg/kg bw). About 0.3 % of the radioactivity remained in the carcasses of the sacrificed animals after 7 days. Thus, in male and female rats almost all the administered radioactivity was excreted via in the urine and faeces within 7 days (97 % and 104 %, 10 mg/kg bw; 105 % and 104 %, 600 mg/kg bw).

Table B.6.1-1: Excretion balance (in mean % of applied dose) up to 168 h post dosing

Balance/Excretion	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
Urine 0-6	2.63	3.25	11.55	9.08
Urine 6-24	15.85	12.69	13.85	13.36
Urine 24-48	2.82	2.41	2.33	4.40
Urine 48-72	0.54	0.44	0.59	1.07
Urine 72-96	0.24	0.19	0.30	0.40
Urine 96-120	0.15	0.13	0.21	0.24
Urine 120-144	0.09	0.07	0.17	0.17
Urine 144-168	0.07	0.05	0.13	0.18
Cage wash	0.12	0.14	1.13	0.60
Subtotal urine + cage wash	22.51	19.37	30.26	29.50
Faeces 0-24	60.28	74.59	58.94	46.28
Faeces 24-48	11.72	7.56	13.41	22.87
Faeces 48-72	1.18	1.34	1.36	3.83
Faeces 72-96	0.29	0.36	0.35	0.47
Faeces 96-120	0.17	0.27	0.36	0.23
Faeces 120-144	0.35	0.08	0.08	0.12
Faeces 144-168	0.64	0.10	0.15	0.35
Subtotal faeces	74.63	84.30	74.65	74.15
Carcass	0.33	0.27	0.31	0.39
Total	97.47	103.94	105.22	104.04

Concentration of radioactivity in the plasma

After a single oral dose of 10 mg/kg bw ¹⁴C HR-001 to rats peak mean concentrations of radioactivity in plasma occurred at 6 and 2 h in males (0.22 µg equiv./mL) and females (0.28 µg equiv./mL), respectively. The absorption rate constants were 0.2963 h⁻¹ in males and 0.4239 h⁻¹ in females. Concentrations declined with an approximate half-life of 8.3 h in males and 7.8 h in females. The area under the concentration *versus* time curve (AUC_t) was 3.2 and 3.7 µg equiv./mL *h in males and females, respectively (see Table B.6.1-2).

After a single oral dose of 600 mg/kg bw ¹⁴C HR-001 to rats peak mean concentrations of radioactivity in plasma occurred at 3 h in males (26 µg equiv./mL) and females (29 µg

equiv./mL), respectively (see Table B.6.1-3). The absorption rate constants were with 0.2845 h⁻¹ in males and 0.4477 h⁻¹ in females comparable with the low dose group, thus absorption did not increase with dose. Concentrations declined with an approximate half-life of 5.9 h in males. The terminal half life could not be calculated for females of the high dose group due to rapid clearance from plasma. The area under the concentration *versus* time curve (AUC) was calculated at 400 and 355 µg equiv./mL*h in males and females, respectively. These values were around 120 fold higher than the AUC_t obtained in the low dose group.

Table B.6.1-2: Kinetic parameters in plasma after single oral doses of 10 or 600 mg/kg bw

	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
C _{max} (µg equiv./mL)	0.2219	0.2789	25.97	28.84
T _{max} (h)	6.00	2.00	3.00	3.00
AUC _t (µg equiv./mL*h)	3.20	3.70	399.90	355.30
AUC (µg equiv./mL*h)	3.80	4.20	419.00	*
Terminal rate constant (h ⁻¹)	0.0840	0.0887	0.1174	*
Terminal half life (h)	8.30	7.80	5.90	*
Absorption rate constant (h ⁻¹)	0.2963	0.4239	0.2845	0.4477

* could not be calculated

Measurements in whole blood in general lead to the same result.

Distribution of radioactivity in tissue

Radioactivity concentrations in tissues were very low at all times (see Table B.6.1-3 and Table B.6.1-4). There was no indication of accumulation of radioactivity in any tissue. Only the gastrointestinal tract (GIT) the stomach muscles and the kidneys, the organs of excretion contained higher concentrations of radioactivity than the plasma. High levels of radioactivity were detected in the content of stomach and GIT. At 7 days p.a. the radioactivity in most tissues had decreased to around the limit of detection. Highest remaining concentrations were detected in carcass (<0.4 %).

Table B.6.1-3: Radioactivity in tissues after single oral dose of 10 mg/kg bw (in mean % of applied dose, except bone and skin expressed as % of applied dose/g)

Tissue	Males			Females		
	6 h (n=3)	18 h (n=3)	168 h (n=5)	6 h (n=3)	18 h (n=3)	168 h (n=5)
Adrenal glands	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bone	0.12	0.10	0.02	0.10	0.09	0.03
Bone marrow	0.01	0.01	<0.01	0.01	0.01	0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carcass	2.00	2.69	0.33	1.69	3.03	0.27
Eyes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fat (abdominal)	0.06	0.04	0.01	0.04	0.03	0.01
Gastrointestinal tract	19.05	10.04	0.01	16.47	5.41	0.01
GIT contents	31.56	4.89	0.01	34.54	14.30	0.01
Heart	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Kidneys	0.79	0.36	<0.01	0.67	0.26	<0.01
Liver	0.07	0.09	0.01	0.06	0.07	0.01
Lungs	0.01	0.01	<0.01	0.01	0.01	<0.01
Lymph nodes	0.09	0.05	<0.01	0.04	0.04	<0.01
Muscle (skeletal)	0.23	0.13	0.04	0.24	0.11	<0.03

Tissue	Males			Females		
	6 h (n=3)	18 h (n=3)	168 h (n=5)	6 h (n=3)	18 h (n=3)	168 h (n=5)
Ovaries	-	-	-	<0.01	<0.01	<0.01
Pancreas	0.02	<0.01	<0.01	0.01	<0.01	<0.01
Pituitary gland	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Plasma	0.12	0.03	<0.01	0.13	0.03	<0.01
Skin	0.01	0.01	<0.01	0.01	0.01	<0.01
Spleen	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Stomach	3.47	0.60	0.60	2.56	0.62	<0.01
Stomach contents	25.16	5.05	0.01	22.90	6.96	0.01
Testes	0.01	0.01	<0.01	-	-	-
Thymus	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thyroid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Urinary bladder	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Uterus	-	-	-	0.01	<0.01	<0.01
Whole blood	0.20	0.04	<0.03	0.15	0.05	<0.03

Table B.6.1-4: Radioactivity in tissues after single oral dose of 600 mg/kg bw (in mean % of applied dose, except bone and skin expressed as mean % of applied dose/g)

Tissue	Males			Females		
	3 h (n=3)	9 h (n=3)	168 h (n=5)	3 h (n=3)	9 h (n=3)	168 h (n=5)
Adrenal glands	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bone	0.09	0.10	0.02	0.09	0.05	0.02
Bone marrow	0.01	0.01	<0.01	0.01	0.01	<0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carcass	1.87	1.70	0.31	2.85	2.41	0.39
Eyes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fat (abdominal)	0.09	0.05	<0.01	0.09	0.02	<0.01
Gastrointestinal tract	19.71	9.99	0.01	20.90	9.33	0.01
GIT contents	30.48	13.9	0.02	22.65	12.86	0.03
Heart	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Kidneys	1.00	0.55	<0.01	0.82	0.21	<0.01
Liver	0.06	0.14	0.01	0.07	0.06	0.02
Lungs	0.02	0.01	<0.01	0.02	0.01	<0.01
Lymph nodes	0.07	0.02	<0.01	0.04	0.01	<0.01
Muscle (skeletal)	0.38	0.19	<0.05	0.48	0.18	<0.05
Ovaries	-	-	-	<0.01	<0.01	<0.01
Pancreas	0.01	0.01	<0.01	0.01	0.01	<0.01
Pituitary gland	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Plasma	0.26	0.07	<0.01	0.30	<0.01	<0.01
Skin	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Spleen	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Stomach	3.53	3.36	<0.01	4.33	3.14	<0.01
Stomach contents	28.73	32.70	0.02	34.20	45.01	0.02
Testes	0.01	0.01	<0.01	-	-	-
Thymus	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thyroid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Urinary bladder	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Uterus	-	-	-	0.01	<0.01	<0.01
Whole blood	0.28	0.08	0.01	0.30	0.05	<0.01

Proportion of radioactive components in urine

Major urinary (18 - 27 %) and faecal (65 - 78 %) component was the parent compound. One further minor component was also observed in urine (0.1 – 0.3 %) and identified as aminomethyl phosphonic acid by TLC and HPLC co-chromatography. In faeces two further minor components were detected (1-2 %, low dose; 0.3-0.6 %, high dose). One of them could be identified as aminomethyl phosphonic acid by TLC and HPLC co-chromatography.

Conclusion by the Notifiers

After oral administration of glyphosate (HR-001) at least about 25 % are absorbed. Absorption was similar in both sexes. About 75 % and 25 % of the parent compound are excreted via faeces and urine, respectively. There was no indication for accumulation of glyphosate.

RMS comments

The study is considered acceptable. As in some other studies of this section, it is not clear if the animals were fasted before sacrifice but this will not have affected the outcome of the study. The results confirmed previous knowledge, in particular with regard to rather poor oral absorption from the gut (based on renal excretion), distribution of radioactivity to the various body compartments with certain affinity to the bones, virtually complete excretion and very minor metabolism. The identity of the second minor component in faeces (beside AMPA) should have been elucidated but it may be assumed to be a transformation product of bacterial activity in the gut. Unfortunately, no experimental group receiving multiple treatment was included in this study. Thus, final proof for the absence of an accumulating potential cannot be taken from the results of this study alone. However, such investigations have been performed by other researchers (██████████, 1996 (TOX2000-1979); ██████████, 1992 (TOX9300343); ██████████ 1988 (TOX9552356)) giving sufficient information on this endpoint.

2nd study (██████████, 1996)

Reference: IIA, 5.1.1/02

Report: ██████████ (1996) [¹⁴C]-Glyphosate:
Absorption, distribution, metabolism and excretion following oral administration to the rat

██████████
Data owner: Nufarm

Report No.: 1413/2-1011

Date: 1996-10-23, not published, ASB2012-11380

Guidelines: Japanese MAFF, 59 NohSan, Notification No. 4200 (1995)

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Materials and methods

Non-labelled test material:

Identification: Glyphosate
Description: Not reported
Lot/Batch #: 08808TG and H95D161A
Purity: 96 % and 95.3 %, respectively
Stability of test compound: Not reported

Radiolabelled test material:

Identification: [¹⁴C] – glyphosate
Position of radiolabel: N-(phosphono[¹⁴C]methyl)glycine
Description: Not reported
Lot/Batch #: 24, lot 3 and 25, lot 4-7
Purity: > 99 % (HPLC and TLC)
Specific activity: 310 µCi/mg, 53 mCi/mmol
Stability of test compound: Stable over 24 h under the conditions of the study

Reference substance:

Identification: Aminomethylphosphonic acid (AMPA) (CAS No. 1066-51-9)
Description: Not reported
Lot/Batch #: 50526010
Purity: Not reported
Stability of test compound: Not reported
Vehicle: Deionised water

Test animals:

Species: Rat
Strain: Sprague-Dawley (CrI:CD BR)
Source: [REDACTED]

Age: 6-10 weeks
Sex: Males and females
Weight at dosing: 179 - 280 g (males) and 167 - 205 g (females)
Acclimation period: Approximately 1 week

Diet/Food: SQC Rat and Mouse Maintenance Diet No. 1, Expanded (Special Diet Services, Stepfield, Witham, Essex, UK), *ad libitum*
Diet was removed the evening before and returned 4 h after administration.

Water: Tap water, *ad libitum*

Housing:

During acclimatisation:

Groups of 5 per cage, in wire floor polypropylene cages suspended over polypropylene dirt trays containing wood saw dust

After dosing:

Excretion-balance experiments - individually in glass metabolism cages

Blood/plasma kinetics - in wire floor cages

Tissue distribution – in wire floor cages

Environmental conditions:

Temperature: 21 ± 2 °C (24 and 26 °C on two consecutive days) This deviation did not affect the study outcome

Humidity: 40 - 70 %

Air changes: not reported

12-hour light/dark cycle

Study design and methods

In life dates: not reported

Animal assignment and treatment: Preliminary excretion study

Four fasted rats (2 males, 2 females) received single oral doses of 100 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 0-12, 12-24, and every 24 h for 7 days in receivers cooled with solid CO₂. Faeces were collected every 24 h for 7 days. Expired air was passed through duplicate traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed after 12, 24, 48 and 72 h after dosing. The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Animal assignment and treatment: Excretion studies

In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study.

Animal assignment and treatment: Plasma concentrations

In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw/day by gavage. Blood samples (0.1 mL) were taken from the tail vein into heparinised tubes at the following times from each animal:

Prior to administration, and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h after administration.

Blood was centrifuged to separate plasma and radioactivity was determined in plasma.

Animal assignment and treatment: Quantitative tissue distribution

In two independent experiments 24 fasted rats (12 male, 12 female) received single oral doses of either 1 or 100 mg/kg bw/day by gavage. The animals were divided into four groups of six (3 per sex) and sacrificed 4, 12, 24 and 72 (low dose) or 4, 6, 24 and 72 h (high dose) after dosing. Animals were exsanguinated under halothane anaesthesia. Following tissues/organs were taken or sampled for radioactivity measurements:

Adrenals, bone, brain, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, muscle (quadriceps), gonads, plasma, skin, spleen, and residual carcass

The contents of the gastrointestinal tract was analysed separately.

Animal assignment and treatment: Biliary excretion study

16 rats were cannulated (8 males, 8 females), of which 14 received single oral doses of 1 mg/kg bw/day by gavage. Following incision animals were placed in metabolism cages and allowed to recover for 24 h. Fully recovered animals were dosed after they were fasted overnight, and bile, urine and faeces were taken from the animals at the following times:

Bile: prior to administration, 0 - 1, 1 - 4, 4 - 6, 6 - 12, 12 - 24 and 24 - 48 h p.a.

Urine, faeces: 0 - 24 and 24 - 48 h p.a. in vessels cooled with solid CO₂.

The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Measurement of radioactivity

Pooled faecal samples were extracted with water prior to solid phase extraction. Urine samples were diluted with water prior to solid phase extraction. Solid phase extraction was performed using columns, conditioned with methanol and de-ionised water. The samples were loaded onto the cartridge washed with de-ionised water, methanol and again water. Radioactivity was eluted using formic acid (5 % v/v). The eluate was freeze dried and reconstituted in water prior to HPLC analysis and where appropriate LC - MS (samples of 100 mg/kg dose group).

A suitable volume of solubilising agent was added to tissue samples. After an appropriate incubation time, liquid scintillant was added and samples were subjected to liquid scintillation counting (LSC). Samples of faecal residues, cage debris homogenates, blood and bone were combusted, absorbed, mixed with scintillation cocktail and analysed by LSC thereafter. Combustion and trapping efficiencies were found to be in excess of 96 % and all reported data are, therefore, uncorrected. Radioassays were performed in duplicates.

Isolation of the major urinary and faecal metabolites

Samples of urine and faecal extracts from male and female rats of the excretion studies were pooled and analysed directly by HPLC. Representative samples were then submitted for analysis by mass spectrometry. The samples were analysed for the presence of glyphosate and the potential metabolite aminomethyl phosphonic acid (AMPA). Following samples were pooled and analysed for each dose group and sex:

Excretion study: Urine 12 - 24 h, Faeces 24 - 48 h

Biliary excretion study: Urine 24 - 48 h, Faeces 24 - 48 h

High performance liquid chromatography (HPLC)

The gradient elution method was used for sample analysis (column: Spherisorp SAX 250 x 4.6 mm id, eluent A: water, eluent B: 0.75 M KH₂PO₄, pH 3.35). The system was linked to a radio-detector. Following HPLC analysis, representative samples were submitted for analysis by mass spectroscopy (samples of 100 mg/kg bw dose group).

Liquid chromatography - Mass Spectrometry (LC - MS)

A VG Quattro triple quadrupole mass spectrometer with electrospray LCMS interface connected to a Jasco ternary gradient HPLC system and a Lablogic β-Ram radio detector were used.

Mode: positive ion electrospray

Scan range: m/z 50 – 250

Mobile phases: water or 1 M formic acid

Glyphosate was detected using Multiple Reaction Monitoring (MRM) of m/z 170 → 88. AMPA was detected using Selected Ion Recording (SIR) of m/z 112.

Results and discussion

Excretion and retention of radioactivity

In a preliminary study with a single dose of 100 mg/kg bw (two rats/sex) the mean total recovery of radioactivity within 7 days was 100.3 % (male) and 95.15 % (female). No relevant radioactivity could be detected in expired air or carcass.

The initial observation was confirmed in the main study with 10 rats per dose (see Table B.6.1-5).

Mean total recovery of radioactivity in rats receiving a single dose of 1 mg/kg bw was 98.31 % in males and 98.81 % in females. Elimination of radioactivity was almost complete within the first 48 h after dosing. The major route of elimination after oral dosing was faeces with 72.62 % and 62.39 % recovered in males and females, respectively, with most of the radioactivity being excreted within the first 24 h after dosing, suggesting this proportion of the dose was not systemically absorbed. During the 7 days observation period 24.92 % (male) and 34.86 % (female) of radioactivity were recovered in the urine, representing the systemically absorbed dose.

After administration of 100 mg/kg bw to rats the mean total recovery of radioactivity was 96.31 % in males and 98.50 % in females. Elimination of radioactivity in the urine (including cage wash 53.27 % in males and 55.04 % in females) was quantitatively more significant compared to the low dose group. Faecal elimination accounted for 41.23 % in males and 42.37 % in females. Again most of the radioactivity was recovered within the first 48 h after dosing.

Table B.6.1-5: Excretion balance (in mean % of applied dose) up to 168 h post dosing

Balance/Excretion	1 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
Urine 0-12	9.52	15.47	31.30	34.93
Urine 12-24	6.14	7.59	4.68	4.46
Urine 24-48	2.10	3.03	2.40	2.32
Urine 48-72	0.35	0.56	0.46	0.71
Urine 72-96	0.15	0.20	0.27	0.33
Urine 96-120	0.09	0.14	0.14	0.15
Urine 120-144	0.06	0.10	0.10	0.10
Urine 144-168	0.04	0.06	0.08	0.07
Cage wash	6.48	7.71	13.85	11.96
Subtotal urine + cage wash	24.92	34.86	53.27	55.04
Faeces 0-24	63.93	49.69	30.46	32.28
Faeces 24-48	7.21	10.93	9.96	4.46
Faeces 48-72	0.65	1.46	0.55	1.10
Faeces 72-96	0.09	0.16	0.12	4.42
Faeces 96-120	0.03	0.06	0.06	0.06
Faeces 120-144	ND	0.07	0.06	0.04
Faeces 144-168	0.71	0.02	0.03	0.01
Subtotal faeces	72.62	62.39	41.23	42.37
Cage debris	0.03	0.58	0.98	0.10

Balance/Excretion	1 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
Carcass	0.75	0.98	0.84	0.98
Total	98.31	98.81	96.31	98.50

Biliary excretion of radioactivity

Biliary excretion was determined in biliary cannulated rats receiving 1 mg/ kg bw. Within 48 h 94.63 % and 95.99 % of radioactivity were recovered in males and females, respectively. Major route of elimination was faeces. Negligible amounts of radioactivity were detected in the bile (see Table B.6.1-6) providing strong evidence that low doses of systemic glyphosate are eliminated almost exclusively in the urine.

Table B.6.1-6: Excretion balance (in mean % of applied dose) at 48 h post dosing in biliary excretion study

	1 mg/kg bw	
	Males	Females
Urine	27.45	24.21
Faeces	55.33	60.97
Bile	0.031	0.076
Cage wash	6.571	6.769
Cage debris	0.262	0.146
Carcass	4.989	3.817
Total	94.63	95.99

Concentration of radioactivity in the plasma

Following a single oral dose of 1 mg/kg bw of the test substance, low levels of radioactivity were detected in plasma (see Table B.6.1-7). Concentrations of radioactivity declined rapidly such that the levels of radioactivity were below the detection limit in most animals by 24 h. The mean terminal elimination half-lives were 10.86 h and 8.07 h with corresponding AUC of 0.319 and 0.340 µg equiv./mL*h in males and females respectively. As the elimination half-lives could not be calculated for several animals of the high dose group mean AUC₀₋₂₄ (0.257 and 0.338 µg equiv./mL*h in males and females) were calculated to compare the results of both groups.

Following a single oral dose of 100 mg/kg bw of the test substance mean maximal plasma concentration of 8.91 (male) and 7.63 µg equiv./mL (female) were observed 2-4 h post-dose in males and 4 h post dose in females (see Table B.6.1-7). Mean AUC₀₋₂₄ were 58.2 and 50.7 µg equiv./mL*h in males and females, respectively. Levels of radioactivity were below the detection limit in males by 48 and in females by 72 h.

Table B.6.1-7: Kinetic parameters in plasma after single oral dose of 1 or 100 mg/kg bw (n = 5)

	1 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
C _{max} (µg equiv./mL)	0.016	0.037	8.909	7.634
T _{max} (h)	3.900	8.000	3.600	4.000
AUC ₀₋₂₄ (µg equiv./mL*h)	0.257	0.338	58.200	50.700
AUC (µg equiv./mL*h)	0.319	0.340	*	*
Terminal half life (h)	10.860	8.065	*	*

* could not be calculated

Distribution of radioactivity in tissue

After administration of 1 mg/kg bw radioactivity concentrations were detected in all tissues by 4 h post-dose (see Table B.6.1-8 and Table B.6.1-9). Apart from the gastrointestinal tract (and content) and carcass, the kidney was the only tissue with a notable content of radioactivity throughout the observation period. By 72 h, post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity. After administration of 100 mg/kg bw all tissues were exposed to radiolabelled material by 4 h post-dose (see Table B.6.1-10 and Table B.6.1-11). Again, only gastrointestinal tract, carcass and kidney contained significant amounts of radioactivity. By 72 h post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity.

Table B.6.1-8: Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
Adrenals	0.014	0.024	0.020	0.009	0.023	0.031	0.022	0.009
Blood	0.010	0.015	0.001	0.002	0.020	0.009	0.002	<0.001
Bone	0.062	0.105	0.201	0.123	0.091	0.140	0.134	0.112
Brain	<0.001	0.003	0.003	0.002	0.002	0.001	0.002	0.002
Carcass	0.021	0.028	0.049	0.016	0.035	0.076	0.045	0.024
Fat	0.022	0.005	0.003	0.002	0.013	0.010	0.006	0.002
GIT + contents	13.040	1.333	1.272	0.026	11.630	3.531	1.314	0.075
Heart	0.006	0.004	0.003	0.002	0.010	0.006	0.004	0.001
Kidney	0.463	0.380	0.307	0.020	0.424	0.387	0.129	0.012
Liver	0.012	0.013	0.022	0.012	0.016	0.018	0.015	0.012
Lung	0.009	0.009	0.013	0.006	0.019	0.013	0.009	0.006
Muscle	0.003	0.001	0.002	<0.001	0.006	0.003	0.002	0.001
Ovaries	-	-	-	-	0.031	0.018	0.021	0.007
Plasma	0.017	0.011	0.006	<0.001	0.027	0.015	0.004	<0.001
Skin	0.010	0.026	0.016	0.006	0.029	0.016	0.106	0.014
Spleen	0.004	0.009	0.010	0.005	0.010	0.009	0.010	0.005
Testes	0.004	0.002	0.001	0.001	-	-	-	-

Table B.6.1-9: Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean % of applied dose)

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
Adrenals	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Brain	<0.001	0.003	0.002	0.002	0.002	0.001	0.002	0.002
Carcass	1.236	1.668	3.048	1.045	1.887	4.115	2.542	1.405
GIT + contents	94.310	17.670	12.990	0.342	89.940	41.740	13.760	0.910
Heart	0.002	0.001	0.001	0.001	0.005	0.003	0.002	0.001
Kidney	0.392	0.304	0.255	0.016	0.348	0.341	0.110	0.011
Liver	0.040	0.050	0.113	0.059	0.055	0.070	0.073	0.057
Lung	0.005	0.005	0.007	0.003	0.012	0.008	0.005	0.004
Ovaries	-	-	-	-	0.002	0.001	0.001	<0.001
Spleen	0.001	0.002	0.002	0.001	0.002	0.002	0.002	0.001
Testes	0.004	0.002	0.002	0.001	-	-	-	-

Table B.6.1-10: Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Adrenals	2.936	5.610	1.856	0.338	8.161	7.244	1.522	0.504
Blood	4.545	4.900	0.016	ND	5.719	1.923	0.218	ND
Bone	24.660	31.360	18.600	11.140	35.450	24.420	17.010	10.420
Brain	0.344	0.699	0.269	0.221	0.619	0.630	0.293	0.215
Carcass	6.097	26.530	4.978	1.843	10.910	38.410	7.206	3.057
Fat	1.366	1.547	0.290	0.120	3.826	2.042	0.393	0.115
GIT+contents	1155.000	544.600	47.750	1.279	1057.000	401.800	59.580	4.320
Heart	2.063	3.424	0.363	0.140	3.704	2.282	0.314	0.092
Kidneys	105.500	127.700	17.440	1.433	132.200	55.770	10.800	1.191
Liver	2.942	4.970	1.831	1.165	5.105	5.564	1.552	0.981
Lung	3.495	4.206	1.069	0.423	6.476	4.623	0.999	0.443
Muscle	0.827	0.887	0.168	0.026	1.698	1.141	0.213	0.051
Ovaries	-	-	-	-	7.532	5.407	1.260	0.438
Plasma	6.479	5.406	0.359	ND	10.830	3.033	0.403	ND
Skin	2.884	3.520	1.293	0.313	6.106	22.480	1.543	0.435
Spleen	1.277	2.678	0.974	0.479	2.337	1.237	0.937	0.395
Testes	0.949	0.942	0.203	0.104	-	-	-	-

ND not detected

Table B.6.1-11: Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean % of applied dose)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Adrenals	0.001	0.001	<0.001	<0.001	0.002	0.001	<0.001	<0.001
Brain	0.003	0.005	0.002	0.002	0.006	0.006	0.003	0.002
Carcass	4.620	8.549	2.402	1.014	8.288	5.879	2.752	1.254
GIT + contents	85.430	64.870	5.456	0.199	75.050	48.910	7.509	0.676
Heart	0.009	0.015	0.001	0.001	0.016	0.009	0.001	<0.001
Kidney	0.870	1.109	0.151	0.012	1.165	0.535	0.096	0.011
Liver	0.104	0.180	0.110	0.060	0.183	0.214	0.088	0.050
Lung	0.027	0.024	0.006	0.003	0.040	0.027	0.007	0.003
Ovaries	-	-	-	-	0.004	0.002	0.001	<0.001
Spleen	0.003	0.007	0.002	0.001	0.006	0.003	0.003	0.001
Testes	0.011	0.010	0.003	0.001	-	-	-	-

Metabolite profiling

After analysis of the pooled samples by HPLC a comparison of chromatograms indicated that the metabolism of the compound was not influenced by the sex or dose level. The peak with the majority of radioactivity could be allocated to [¹⁴C]-glyphosate standard. A peak with <1 % of the total radioactivity was thought to correspond to AMPA. The presence of glyphosate could be confirmed by mass spectroscopy, whereas the presence of AMPA could not be verified due to technical problems.

Conclusion by the Notifiers

After oral administration of glyphosate absorption, distribution, metabolism and excretion were independent of dose level and sex. Absorption was limited and distribution was rapid and extensive. Metabolism was negligible. Elimination was essentially complete within 48 h, with the majority of radioactivity recovered in faeces, likely being the unabsorbed dose. The

remaining radioactivity was excreted with the urine. There was no indication for accumulation of glyphosate.

RMS comments

The study is considered acceptable. Again, poor absorption at least of the low dose, wide distribution and fast excretion of the compound as well as the virtual absence of metabolism were confirmed. Limited oral absorption rate was further supported by the very low amount of biliary excretion that was seldom investigated in ADME studies with glyphosate. In contrast, as compared to other studies, urinary elimination following the high dose was strikingly high. A much higher percentage of radioactivity than in other studies was found in cagewash and may be summed up with the material excreted in urine. The rather high organ/tissue residues in bile-cannulated rats at termination are due to the shorter duration of this experiment and are equal to what was measured in non-cannulated rats after 24 or 72 hours. It should be emphasised that the by far highest residues were found in bone since this was not mentioned in the GTF dossier. Elimination of bone-bound residues was apparently slower than of the radioactivity that had been retained in other tissues.

Unfortunately, no experimental group receiving multiple treatment was included in this study. Thus, final proof for the absence of an accumulating potential cannot be taken from the results of this study alone. However, such investigations have been performed by other researchers [REDACTED] 1996 (TOX2000-1979); [REDACTED] 1992 (TOX9300343); [REDACTED] 1988 (TOX9552356)) giving sufficient information on this endpoint.

The following studies (Davies, 1996 (TOX2000-1977, TOX2000-1978, TOX2000-1979, TOX2000-1980), [REDACTED], 1996 (TOX2000-1981)) have been performed to address certain aspects of toxicokinetics and metabolism of glyphosate. Taken together, they allow comprehensive evaluation.

3d study ([REDACTED], 1996)

Reference: IIA, 5.1.1/03

Report: [REDACTED] 1996) Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat

Data owner: Syngenta

Report No.: [REDACTED]/4940

Date: 1996-04-26, not published, TOX2000-1977

Guidelines: MAFF (Japan) Metabolism Study (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)

Deviations: None

GLP: Yes

Acceptability: See RMS comment.

Materials and methods

Non-labelled test material:	Glyphosate acid
Identification:	N-phosphonomethyl glycine
Description:	White solid
Lot/Batch #:	Y04707/045
Purity:	99.2 %
Stability of test compound:	Stable throughout the experiment
Radiolabelled test material:	
Identification:	[¹⁴ C]–glyphosate acid
Position of radiolabel:	N-(phosphono[¹⁴ C]methyl)glycine
Lot/Batch #:	Y04707/047
Purity:	> 98 %
Specific activity:	1.580 GBq/mMol
Stability of test compound:	Stable throughout the experiment
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Alpk:AP _f SD
Source:	
Age:	Not reported
Sex:	Male/female
Weight at dosing:	195 – 235 g
Acclimation period:	At least 5 days
Diet/Food:	PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	During acclimatisation: Groups of the same sex, in stock rat cages, 24 h prior to dosing transfer individually into metabolism cages After dosing: Excretion-balance experiments - individually in metabolism cages
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: 12/hour 12-hour light/dark cycle

Study design and methods

In life dates: not reported

Animal assignment and treatment: (Excretion study and quantitative tissue distribution)

Ten rats (5 male, 5 female) received a single oral dose of 10 mg [¹⁴C]-glyphosate acid/kg bw/day (10 mL/kg, 0.6 MBq/kg of dosing solution) by gavage and were placed back in glass

metabolism cages immediately thereafter. Urine was collected at 6, 12, 24, 36, 48 and 72 h after dosing in receivers cooled with solid CO₂. Faeces were collected at 12, 24, 36, 48 and 72 h. The interior of the cages were washed with water after each collection time. At the end of the study cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20 °C until taken for analysis.

Animals were exsanguinated by cardiac puncture under halothane anaesthesia. Two blood samples were collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements: bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, heart, kidneys, liver, lungs, muscle (femoral), gonads, spleen, salivary glands, residual carcass.

Measurement of radioactivity

Samples of urine, cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus contents were homogenised. Liver, fat and residual carcass were then solubilised in Soluene-350[®] whereas GIT plus contents were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation

Samples were oxidised in a Packard Tricarb sample oxidiser. The [¹⁴C]-carbon dioxide generated was absorbed into Carbo-sorb E[®] and mixed with Permafluor E+[®] scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)

Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3[®] and counted for [¹⁴C]-radioactivity to a 1% standard deviation of the count or for a maximum of 10 min, whichever occurred first. The results obtained were corrected for background activity and counting efficiency using [¹³⁵Ba] as the external source.

Results and discussion

Excretion and retention of radioactivity

After a single oral dose to rats, excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (means of 77.8 % in males, and 80.7 % in females). In the urine, means of 11.5 % and 9.4 % of the radioactivity were eliminated in the first 24 h in male and female rats, respectively. Within the observation period of 72 h, means of 101.8 % (male) and 99.6 % (female) of the administered radioactivity were excreted (see Table B.6.1-12). There were no differences in the cumulative excretion patterns between the sexes.

Table B.6.1-12: Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	10 mg/kg bw (oral gavage)	
	Males	Females
Urine 0-6	3.7	3.5
Urine 6-12	4.5	3.3
Urine 12-24	3.3	2.6
Urine 24-36	0.8	0.7
Urine 36-48	0.4	0.4
Urine 48-72	0.3	0.2
Cage wash	0.3	0.4
Subtotal urine + cage wash	13.3	11.1
Faeces 0-12	42.3	48.1
Faeces 12-24	35.5	32.6
Faeces 24-36	6.6	3.9
Faeces 36-48	2.8	2.9
Faeces 48-72	1.3	1.2
Subtotal faeces	88.5	88.7
Total	101.8	99.6

Distribution of radioactivity in tissues

The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 0.51 µg equiv./g (male) and 0.40 µg equiv./g (female), followed by the intestinal tract plus contents with 0.15 µg equiv./g (males and females). Lower mean concentrations between 0.07 and 0.01 µg equiv./g were found in kidneys, liver, lungs, spleen, salivary glands and ovaries. Mean concentrations of 0.06 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of either sex. All other concentrations were either similar to or lower than the corresponding blood concentrations (see Table B.6.1-13). The mean total percentage of administered radioactivity present in all of the tissues examined and the residual carcass was 0.6 % for males and 0.5 % for females. The amounts in the intestinal tract plus contents were about 0.2 % for both sexes.

Table B.6.1-13: Radioactivity in tissues after a single oral dose of 10 mg/kg bw at 72 h

Tissue	Males		Females	
	% of dose	µg equiv./g	% of dose	µg equiv./g
Blood	N/A	0.011	N/A	0.009
Bone (femur)	N/A	0.511	N/A	0.395
Brain	0.001	0.011	0.001	0.009
Fat (abdominal)	N/A	0.007	N/A	<0.004
Heart	<0.001	0.012	<0.001	0.011
Kidneys	0.007	0.068	0.004	0.049
Liver	0.036	0.059	0.022	0.044
Lungs	0.002	0.031	0.001	0.026
Muscle (femoral)	N/A	0.007	N/A	0.006
Ovary	-	-	<0.001	0.024
Plasma	N/A	N/A	N/A	<0.004
Residual Carcass	0.542	0.062	0.458	0.056
Salivary glands	<0.001	0.017	<0.001	0.018
Spleen	0.001	0.026	0.001	0.024
Testes	0.001	0.007	-	-
Total	0.590	N/A	0.488	N/A
GIT plus contents	0.186	0.152	0.172	0.152

N/A not applicable

Conclusion by the Notifiers

After a single oral dose glyphosate acid was excreted rapidly and predominantly in faeces. Elimination was essentially complete within 72 h. There was no indication for accumulation of glyphosate acid.

RMS comments

The study is considered acceptable. Sufficient data was provided to describe toxicokinetics and tissue residues of glyphosate following administration of a low dose of 10 mg/kg bw. Plasma kinetics was not followed and metabolism was not investigated but enough information on these parameters is available from other sources.

Taking into account the negligible biliary excretion (██████████ 1996 (ASB2012-11380); ██████████, 1996 (TOX2000-1981)), oral absorption rate is estimated on the basis of renal elimination. As compared to other studies in which the same dose was applied (██████████, 1995 (ASB2012-11379); ██████████ 1988 (TOX9552356)), urinary excretion in this study accounted for only 50 to 60% in both sexes. The reason for this difference is not known but the lower figure established by ██████████ (1996, TOX2000-1977) must not be ignored when correction of the AOEL for an internal dose is considered.

Affinity of the compound to bone tissue was confirmed once more.

4th study (██████████ 1996)

Reference: IIA, 5.1.1/04

Report: ██████████ (1996) Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat

Data owner: Syngenta

Report No.: ██████████

Date: 1996-06-19, not published, TOX2000-1978

Guidelines: MAFF (Japan) Metabolism Study (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)

Deviations: None

GLP: yes

Acceptability: See RMS comment

Materials and methods

Non-labelled test material: Glyphosate acid

Identification: N-phosphonomethyl glycine

Description: White solid

Lot/Batch #: Y04707/048

Purity: 99.5 %

Stability of test compound: Stable throughout the experiment

Radiolabelled test material:

Identification: [¹⁴C]–glyphosate acid

Position of radiolabel: N-(phosphono[¹⁴C]methyl)glycine

Lot/Batch #: Y04707/047

Purity: > 98 %

Specific activity: 1.580GBq/mMol

Stability of test compound: Stable throughout the experiment

Vehicle and/or positive control: Deionised water

Test animals:

Species: Rat

Strain: Alpk:AP_fSD

Source: [REDACTED]

Age: Not reported

Sex: Male/female

Weight at dosing: 182 - 235 g

Acclimation period: At least 4 days

Diet/Food: PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), *ad libitum*

Water: Tap water, *ad libitum*

Housing: During acclimatisation:
Groups of 6 per cage and sex, in stock rat cages
After dosing:
Excretion-balance experiments - individually in metabolism cages

Environmental conditions: Temperature: 21 ± 2 °C
Humidity: 40 - 70 %
Air changes: 12/hour
12-hour light/dark cycle

Study design and methods

In life dates: not reported

Animal assignment and treatment (Excretion study and quantitative tissue distribution)

Ten non-fasted rats (5 male, 5 female) received single oral doses of 1000 mg/kg bw/day (10 mL/kg, 6MBq/kg) by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 6, 12, 24, 36, 48 and 72 h after dosing in receivers cooled with solid CO₂. Faeces were collected at 12, 24, 36, 48 and 72 h. The interior of the cages were washed with water after each collection time. At the end of the study cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20 °C until taken for analysis.

Animals were exsanguinated by cardiac puncture under halothane anaesthesia. Two blood samples were collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements:

Bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, heart, kidneys, liver, lungs, muscle (femoral), gonads, spleen, salivary glands, residual carcass.

Measurement of radioactivity

Samples of urine cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus contents were homogenised. Liver and fat were then solubilised in Soluene-350[®] whereas

GIT and residual carcass were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation

Samples were oxidised in a Packard Tricarb sample oxidiser. The [^{14}C]-carbon dioxide generated was absorbed into Carbo-sorb E[®] and mixed with Permafluor E+[®] scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)

Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3[®] and counted for a maximum of 10 min in Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficiency using [^{135}Ba] as the external source.

Results and discussion

Excretion and retention of radioactivity

Excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (78.7 % in males, and 71.3 % in females). In the urine, means of 15.3 % and 16.0 % of the radioactivity were eliminated in the first 24 h in males and females, respectively. Within the observation period of 72 h, means of 106.4 % (male) and 102.3 % (female) of the administered radioactivity were excreted in total (see Table B.6.1-14).

Table B.6.1-14: Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	1000 mg/kg bw	
	Males	Females
Urine 0-6	7.9	9.7
Urine 6-12	5.0	3.9
Urine 12-24	2.5	2.4
Urine 24-36	0.7	0.8
Urine 36-48	0.4	0.5
Urine 48-72	0.3	0.3
Cage wash	0.1	0.2
Subtotal urine + cage wash	16.9	17.8
Faeces 0-12	36.4	19.7
Faeces 12-24	42.2	51.6
Faeces 24-36	6.6	8.5
Faeces 36-48	2.9	3.5
Faeces 48-72	1.4	1.3
Subtotal faeces	89.5	84.6
Total	106.4	102.4

Distribution of radioactivity in tissues

The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 49.8 µg equiv./g (male) and 44.9 µg equiv./g (female), followed by the gastrointestinal tract (and contents) with 13.3 µg equiv./g (male and 16.3 µg equiv./g (female). Lower mean concentrations between 6.6 and 1.1 µg equiv./g were found in kidneys, liver, heart, lungs, spleen, brain, gonads and salivary glands of both sexes. Mean concentrations of 4.8 and 5.9 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of males and females respectively. All other concentrations were either similar to or lower than the corresponding blood concentrations (see Table B.6.1-15).

Table B.6.1-15: Radioactivity in tissues after single oral dose of 1000 mg/kg bw at 72 h

Tissue	Males		Females	
	% of dose	µg equiv./g	% of dose	µg equiv./g
Blood	N/A	0.894	N/A	0.803
Bone	N/A	49.792	N/A	44.925
Brain	0.001	1.233	0.001	1.164
Fat	N/A	0.536	N/A	0.496
GIT plus contents	0.2	13.276	0.219	16.329
Heart	0.001	1.111	0.001	1.254
Kidneys	0.007	6.511	0.005	6.046
Liver	0.039	5.480	0.029	5.226
Lungs	0.002	2.870	0.002	3.535
Muscle	N/A	0.816	N/A	0.825
Ovary	-	-	<0.001	2.940
Plasma	N/A	<0.392	N/A	<0.396
Residual carcass	0.466	4.772	0.537	5.858
Salivary glands	<0.001	1.811	<0.001	2.089
Spleen	0.001	2.441	0.001	3.106
Testes	0.001	0.905	-	-

N/A not applicable

Conclusion by the Notifiers

Oral doses of glyphosate acid were excreted rapidly and predominantly in the faeces. Elimination was essentially complete within 48 h. The remaining radioactivity was excreted with the urine. Negligible traces of radioactivity (<0.6 %) were still present in the tissues and residual carcass at 72 h, with bone representing the highest tissue residue. Thus, there was no indication for accumulation of glyphosate acid.

RMS comments

The study is considered acceptable. The conclusion is agreed with. Again, oral absorption rate (expressed as percentage of urinary excretion) was rather low in this study but there is no direct comparison with other studies from other laboratories possible since 1000 mg/kg bw was by far the highest dose that was administered in ADME studies. However, the urinary excretion rates were in line with data obtained by [REDACTED] (1996, TOX2000-1981).

Plasma kinetics was not followed and metabolism was not investigated but enough information on these parameters is available from other sources.

The tissue distribution pattern observed by other researchers was confirmed.

5th study ([REDACTED] 1996)**Reference:** IIA, 5.1.3/03**Report:** [REDACTED] (1996) Glyphosate acid: Biotransformation in the rat

Data owner: Syngenta

Report No.: [REDACTED]/P/5058

Date: 1996-06-28, not published, TOX2000-1981

Guidelines: OECD 417 (1984)**Deviations:** None**GLP:** Yes**Acceptability:** See RMS comment

Materials and methods

Non-labelled test material:	Glyphosate acid
Identification:	N-(phosphonomethyl) glycine
Description:	White solid
Lot/Batch #:	Y04707/048
Purity:	99.5 % w/w
Stability of test compound:	Not reported
Radiolabelled test material:	[¹⁴ C]-phosphonomethyl-labelled glyphosate acid
Identification:	[¹⁴ C]-phosphonomethyl-labelled glyphosate acid
Description:	Not reported
Lot/Batch #:	Y04707/047
Purity:	97.8 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	The test substance was shown to be stable in vehicle for longer than the period of use during this study.
Reference substance:	
Identification:	Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported
Test animals:	
Species:	Rat
Strain:	AlpkAP _f SD
Source:	
Age:	Not reported
Sex:	Males and females
Weight at dosing:	260- 305 g
Acclimation period:	At least 4 days in stock rat cages and 24 hours prior to surgery in metabolism cages
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	Housed individually in glass metabolism cages
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: At least 12 change/hour 12-hour light/dark cycle

Study design and methods

In life dates: 1995-11-26 to 1996 May

Animal assignment and treatment

Two male and two female non-fasted rats were administered with a single oral dose of 1000 mg [^{14}C]-glyphosate acid/kg by gavage after bile duct cannulation. 48 hours after dosing all animals were sacrificed.

Bile duct cannulation

The abdominal cavity was opened after anaesthesia and the bile duct exposed. A fine plastic cannula was inserted into the bile duct and externalised by passing through the abdominal wall and under the skin to an exit point at the back of the neck. The incisions in the abdominal and body walls were saturated and the exposed cannula was protected within a flexible metal sheath anchored to the skin at the back of the neck. Following surgery each animal was returned to its cage and allowed to recover overnight prior dosing.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The radiochemical purity of the [^{14}C] labelled test substance was determined by the high performance liquid chromatography (HPLC) following formulation in the dosing.

Collection of excreta and bile

Urine only was collected 6 hours after dosing, and separately together with faeces 12, 24, 36 and 48 hours after dosing from all surviving animals.

Bile was collected at 2, 4, 6, 8, 12, 24, 36 and 48 hours after dosing.

Faecal samples were analysed by sample oxidation followed by liquid scintillation counting whereas samples of urine and bile were analysed without intermediate processing.

Quantification of metabolites

Urine and faecal samples obtained from previous excretion and tissue distribution studies (Davies 1996 TOX2000-1977, TOX2000-1978, TOX2000-1979) over 72 hours were used for the quantification of metabolites.

Urine samples were combined by taking a fixed percentage by weight to give separate male and female pools for each of the sample collections intervals. Subsamples of these pools were further combined to give pools representing the entire sample collection period. Each pool was analysed by TLC and HPLC. A representative urine sample was analysed by ^1H -NMR.

Faecal samples were combined in the same way as described above for urine samples. Subsamples of pooled faecal samples were mixed with distilled water and sonicated for several hours, the samples were filtered through filter paper and the solid material was re-extracted a second time with distilled water and a third time with 10% aqueous HCl. Extract volumes were measured and aliquots taken for scintillation counting to allow the calculation of extraction efficiencies.

Measurement of radioactivity

Liquid scintillation counting

Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.

Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel (60F₂₅₄) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15: 15 v/v/v/v).

Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser or a Bioscan System 200 imaging scanner. Glyphosate acid and AMPA standards were located by spraying the plates with a solution of 300 mg ninhydrin in 100 mL of butanol and 3 mL of glacial acetic acid.

High performance liquid chromatography (HPLC)

Two different HPLC methods were employed:

HPLC method 1 was used for the analysis of dosing solutions. Prior to analysis samples were derivatised. A Hichrom S5NH column (250 x 4.6 mm) was eluted with acetonitrile buffered with 25 mMol aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min.

HPLC method 2 was used for the analysis of urine and faecal extracts and for the quantification of glyphosate acid and AMPA. Prior to analysis samples were filtered. A Biorad's HLRC acid analysis column (250 x 4.6 mm) was eluted with 5 mM aqueous potassium dihydrogen phosphate with 4 % methanol at a flow rate of 0.5 mL/min.

Radioactivity was detected in both methods by liquid cell.

Proton Nuclear Magnetic Resonance Spectroscopy (¹H-NMR)

Proton and phosphorus NMR spectra were acquired using a Bruker 400MHz instrument. Samples of glyphosate acid and AMPA were dissolved in D₂O and analysed by both phosphorus and proton NMR. Control urine and urine from a bile duct cannulated rat administered an oral dose of glyphosate acid were analysed by phosphorus NMR. The urine sample from the rat that had been administered glyphosate was subsequently fortified with AMPA then glyphosate acid and reanalysed by phosphorus NMR.

Data evaluation

Dosing and excretion of radioactivity data were processed using the Debra computerised acquisition and processing system. Metabolites were quantified using the Flo_One integration software for HPLC.

Results and discussion

Excretion of radioactivity

The results showed that 48 hours after dosing, excreted means of the administered dose in the urine and faeces amounted to 20.8 % and 39.1 % in males and 16.3 % and 30.5 % in females, respectively, whereas biliary excretion of radioactivity was negligible (see Table B.6.1-16).

The total excreted radioactivity after 48 hours accounted for 62.5 % and 52.0 % in males and females, respectively.

Table B.6.1-16: Excretion of radioactivity in urine, faeces and bile by male and female bile duct cannulated rats given a single oral dose of 1000 mg [¹⁴C] glyphosate acid/kg bw (mean of two rats expressed as % of applied dose)

Time after dosing (hours)	Males rats			Female rats		
	% of applied dose			% of applied dose		
	Urine	Faeces	Bile	Urine	Faeces	Bile
0-2	N/A	N/A	0.004	N/A	N/A	0.002
2-4	N/A	N/A	0.004	N/A	N/A	0.011
4-6	N/A	N/A	0.002	N/A	N/A	0.011
0-6	2.137	N/A	N/A	8.718	N/A	N/A
6-8	N/A	N/A	0.005	N/A	N/A	0.005
0-12	N/A	3.776	N/A	N/A	1.392	N/A
6-12	6.765	N/A	N/A	2.495	N/A	N/A
8-12	N/A	N/A	0.008	N/A	N/A	0.007
12-24	5.432	12.333	0.016	3.631	12.115	0.010
24-36	3.468	18.079	0.009	1.004	8.712	0.008
36-48	3.013	4.946	0.007	0.427	8.325	0.007
0-48	20.815	39.134	0.055	16.275	30.544	0.062
Cage wash at 48 hours	2.534 (mean)			5.097 (mean)		
Total excreted	62.538			51.978		

Values are expressed as percentages of administered dose and are then mean of two rats

Characterisation of radioactivity

The negligible levels of radioactivity in bile samples made chromatographic analysis unnecessary. Corresponding to this result, faecal extracts analysed by HPLC and NMR confirmed the radioactivity as glyphosate acid.

Analyses by chromatography and phosphorus NMR of urine pools from former studies (1996, TOX2000-1977, TOX2000-1978, TOX2000-1979) covering the 0-72 hour period demonstrated a single peak identified as glyphosate acid. Earlier timepoints demonstrated a second peak identified as aminomethyl phosphonic acid (AMPA) that occurred in measurable quantities.

The percentages of dose accounted for glyphosate acid and AMPA following a low, high or repeated dose of glyphosate acid are given in Table B.6.1-17. For glyphosate acid and AMPA, the values range from 63.3-95.3 % and 0.07-0.66 %, respectively.

Table B.6.1-17: Percentage of administered radioactivity identified as glyphosate acid and AMPA

		Low dose study 10 mg/kg bw		High dose study 1000 mg/kg bw		Repeated dose study 10 mg/kg bw	
		Male	Female	Male	Female	Male	Female
		(% of applied radioactivity)					
Urine	Glyphosate acid	12.71	10.51	16.00	16.73	10.46	10.47
	AMPA	0.19	0.11	0.63	0.66	0.07	0.08
Faeces	Glyphosate acid	74.80	55.22	79.25	63.88	52.86	72.09
Total	Glyphosate acid	87.52	65.73	95.25	80.61	63.33	82.57
	AMPA	0.19	0.11	0.63	0.66	0.07	0.08

Conclusion by the Notifiers

Following an oral dose of glyphosate acid to rats approximately 10-20 % of the dose was absorbed. The unabsorbed glyphosate acid was excreted unchanged in faeces. The absorbed dose was excreted in urine as glyphosate and trace amounts of aminomethyl phosphonic acid (AMPA).

RMS comments

The study is considered acceptable. Data obtained on bile-cannulated rats and on metabolism amend the information provided by Davies (1996, TOX2000-1977, TOX2000-1978) and confirm low urinary excretion (although some underestimation is possible since 2-5 – 5 % radioactivity in cage was not included), negligible biliary excretion also following administration of a high dose and virtually absent metabolism.

6th study (1996)**Reference:**

IIA, 5.1.3/01

Report:

(1996) Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing

Data owner: Syngenta

Report No.: /P/4944

Date: 1996-05-22, not published, TOX2000-1979

Guidelines:

OECD 417 (1984)

Deviations:

None

GLP:

Yes

Acceptability:

See RMS comment

Materials and methods

Non-labelled test material:	Glyphosate acid
Identification:	N-phosphonomethyl glycine
Description:	White solid
Lot/Batch #:	Y04707/045
Purity:	99.2 % w/w
Stability of test compound:	Not reported
Radiolabelled test material:	[¹⁴ C]-phosphonomethyl-labelled glyphosate acid
Identification:	[¹⁴ C]-phosphonomethyl-labelled glyphosate acid
Description:	Not reported
Lot/Batch #:	Y04707/047
Purity:	> 98 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	The test substance was shown to be stable in the vehicle for longer than a period of use during the study.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Alpk:AP _f SD
Source:	[REDACTED]
Age:	Not reported
Sex:	Males and females
Weight at dosing:	225- 328 g
Acclimation period:	At least 4 days prior to the study start and 24 hours prior to dosing with the radiolabelled preparation
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Wiltham, Essex, UK), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	After administration of the 14 th unlabelled dose: Individually in stainless steel metabolism cages
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: At least 12 change/hour 12-hour light/dark cycle

Study design and methods

In life dates: 1995-10-16 to 1996-03-26

Animal assignment and treatment

Eight male and eight female non-fasted rats received a single oral dose of the unlabelled test substance (10 mg/kg) daily for 14 days by gavage. 24 hours after the 14th dose of unlabelled glyphosate acid, five male rats and five female rats were given a single oral dose (10 mg/kg)

of [^{14}C]-phosphonomethyl glyphosate acid. 72 hours after dosing, the rats were killed and the residual radioactivity was measured in blood, selected tissues and in the residual carcasses.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) high performance liquid chromatography (HPLC).

Collection of excreta

Urine only was collected at 6 hours after dosing, while urine together with faeces were separately collected at 12, 24, 36, 48 and 72 hours after dosing.

Urine collections comprised rinsing of each cage at each time point together with a thorough washing at the end of the study. Faecal samples were analysed by sample oxidation followed by liquid scintillation counting whereas samples of urine and cage washings were analysed without intermediate processing.

Collection of blood and tissues

72 hours after dosing, the rats were sacrificed and a blood sample was retained and divided into two portions. A portion of each blood sample was centrifuged to obtain plasma, which was analysed for liquid scintillation counting. Whole blood was analysed by sample oxidation.

The following tissues together with representative samples of fat (abdominal), bone (femur), and muscle (femoral) were removed from each rat: brain, liver, testes or ovaries, lungs, heart, spleen, kidneys, salivary glands, intestinal tract plus contents and residual carcasses.

Measurement of radioactivity - Liquid scintillation counting

Radioactivity was measured by liquid scintillation counting by means of Packard Tricarb instruments. The results obtained from the counting were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.

Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel (60F₂₅₄) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15:5 v/v/v/v).

Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.

High performance liquid chromatography (HPLC)

To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mMol aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min. Radioactivity was detected using an on-line flow detector (liquid cell) and with UV absorption at 230 nm.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample which was twice the liquid scintillation counter's background rate. For the purpose of calculating group mean results, individual values below the LOD are accepted as being equal to the limit of detection. Means which include one or more values which are below the LOD are reported as "<" the mean result and without a standard deviation. The limit of detection obtained for all tissues in this study was 0.004/ μ g equivalents glyphosate acid/g of tissue (μ g equiv/g). This value is based upon a sample size of 200 mg of all determinations. Organs of less than this weight were analysed as a single sample and hence this figure represent a limiting value.

Results and discussion

Excretion of radioactivity

The results showed that the excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 hours after dosing (average of 80.6 % for males and 85.8 % for females).

Excretion of radioactivity in the urine during this period accounted for means of 9.2 % and 9.1 % of the administered dose in the male and female rats respectively. The total percentage of the administered radioactivity eliminated in excreta 72 hours after dosing were means of 97.5 % for males and 101.7 % for females (Table B.6.1-18)

Comparison of the cumulative excretion data showed that there were no marked differences in the rates of excretion of radioactivity in the urine or faeces for male and female rats.

Table B.6.1-18: Excretion of radioactivity in urine and faeces in male and female rats

Time after dosing (hours)	Excretion of radioactivity [%]			
	Males			
	Urine		Faeces	
	Mean ^a	SD	Mean	SD
0-6	3.1	0.8	N/A	N/A
6-12	2.7	0.7	N/A	N/A
0-12	N/A	N/A	50.2	15.5
12-24	3.4	1.6	30.3	9.0
24-36	0.9	0.3	3.6	1.5
36-48	0.3	<0.1	1.3	0.7
48-72	0.2	<0.1	1.1	0.6
0-72	10.6	3.0	86.6	5.2
	Mean	SD	N/A	N/A
Cage wash at 72 h	0.2	<0.1	N/A	N/A
Total excreted	97.5	2.7	N/A	N/A

	Excretion of radioactivity [%]			
	Females			
Time after dosing (hours)	Urine		Faeces	
	Mean ^b	SD	Mean	SD
0-6	3.3	0.6	N/A	N/A
6-12	2.5	0.3	N/A	N/A
0-12	N/A	N/A	44.7	34.1
12-24	3.2	0.6	41.0	31.1
24-36	0.9	0.3	2.7	1.1
36-48	0.4	0.2	1.2	0.2
48-72	0.3	0.1	1.1	0.7
0-72	10.7	1.1	90.7	4.2
	Mean	SD	N/A	N/A
Cage wash at 72 h	0.2	<0.1	N/A	N/A
Total excreted	101.7	4.0	N/A	N/A

N/A not applicable

^a Mean of 4 animals^b Mean of 5 animals

Tissue distribution of radioactivity

The highest tissue concentration of radioactivity was found in bone with a mean concentration of 0.36 µg equiv/g for males and 0.35 µg equiv/g for females, followed by the intestinal tract plus contents, kidneys, liver, lungs, salivary glands, and ovaries.

Mean concentrations of 0.05 µg equiv/g were found in the residual carcass of either sex which also includes the remaining skeletal bone.

All other concentrations were either similar to or lower than the corresponding blood concentrations.

The mean total percentage of administered radioactivity present in all of the tissues examined and the residual carcass was 0.5 % for males and 0.4 % for females. The amounts present in the intestinal tract plus contents were 0.1 % for males and females (see Table B.6.1-19)

Table B.6.1-19: Tissue and carcass residues of radioactivity in male and female rats

	Residue of radioactivity			
	Males			
Tissue	% radioactivity of dose		µg equivalents/g	
	Mean ^a	SD	Mean	SD
Brain	0.001	<0.001	0.010	0.002
Testes	0.001	<0.001	0.007	0.001
Heart	<0.001	<0.001	0.011	0.002
Kidneys	0.005	0.002	0.061	0.015
Liver	0.031	0.009	0.055	0.014
Lungs	0.001	<0.001	0.026	0.004
Spleen	0.001	<0.001	0.022	0.003
Salivary glands	<0.001	<0.001	0.019	0.004
Bone (femur)	N/A	N/A	0.358	0.177
Fat (abdominal)	N/A	N/A	0.008	0.001
Muscle (femoral)	N/A	N/A	0.008	0.001
Blood	N/A	N/A	0.014	0.006
Plasma	N/A	N/A	<0.004	
Residual carcass	0.423	0.090	0.050	0.011
Total	0.463	0.101	N/A	N/A
Intestinal tract plus contents	0.108	0.040	0.109	0.041

Tissue	Residue of radioactivity			
	Females			
	% radioactivity of dose		µg equivalents/g	
	Mean ^b	SD	Mean	SD
Brain	0.001	<0.001	0.010	0.002
Ovaries	<0.001	<0.001	0.026	0.006
Heart	<0.001	<0.001	0.012	0.004
Kidneys	0.004	0.001	0.049	0.011
Liver	0.021	0.005	0.045	0.010
Lungs	0.001	<0.001	0.029	0.006
Spleen	0.001	<0.001	0.025	0.006
Salivary glands	<0.001	<0.001	0.027	0.006
Bone (femur)	N/A	N/A	0.345	0.081
Fat (abdominal)	N/A	N/A	0.006	0.002
Muscle (femoral)	N/A	N/A	0.007	0.002
Blood	N/A	N/A	0.010	0.002
Plasma	N/A	N/A	<0.005	
Residual carcass	0.382	0.067	0.046	0.008
Total	0.411	0.073	N/A	N/A
Intestinal tract plus contents	0.115	0.014	0.117	0.015

N/A not applicable

Residual carcass values include partial tissue percentages

^a Mean of 4 animals

^b Mean of 5 animals

Recovery of radioactivity

The total mean percentage recoveries, including excreta, tissues and residual carcass was 98.0 % for male rats and 102.2 % for females.

Conclusion by the Notifiers

Comparison of the results with those obtained at the same dose level but without pre-administration of unlabelled test substance (██████████ 1996, TOX2000-1977) showed no significant differences on either the routes or rates of elimination after oral dosing. In both studies the test substance was excreted rapidly and predominantly in the faeces by rats of both sex and low amounts of radioactivity were detected in all the tissue examined.

RMS comments

The study is considered acceptable. No significant differences were observed when the results were compared to the data obtained following single administration. In particular, there was no evidence of accumulation when, e.g., the tissue residues were considered. Urinary excretion that may be taken as surrogate for oral absorption was even lower than in the single-dose experiments without pre-treatment, and so was radioactivity in cage wash.

7th study (██████████ 1996)

Reference:

IIA, 5.1.3/02

Report:

██████████ (1996) Glyphosate acid: Whole body autoradiography

Data owner: Syngenta

Report No.: ██████████/P/4943

Date: 1996-06-10, not published, TOX2000-1980

Guidelines: OECD 417 (1984)
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Materials and methods

Non-labelled test material: Glyphosate acid
 Identification: N-phosphonomethyl glycine
 Description: White solid
 Lot/Batch #: Y04707/045
 Purity: 99.2 % w/w
 Stability of test compound: Not reported
 Radiolabelled test material: [¹⁴C]-phosphonomethyl-labelled glyphosate acid
 Identification: [¹⁴C]-phosphonomethyl-labelled glyphosate acid
 Description: Not reported
 Lot/Batch #: Y04707/047
 Purity: > 96 %
 Specific activity : 1.580 GBq/mMol
 Stability of test compound: The test substance was shown to be stable in the vehicle for longer than a period of use during the study
 Vehicle and/or positive control: Deionised water
 Test animals:
 Species: Rat
 Strain: Alpk₀AP_fSD
 Source: [REDACTED]
 Age: Not reported
 Sex: Males and females
 Weight at dosing: 215- 271 g
 Acclimation period: At least 5 days in stock rat cages and 24 hours prior to dosing in metabolism cages
 Diet/Food: Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*
 Water: Tap water, *ad libitum*
 Housing: Housed individually in glass metabolism cages
 Environmental conditions: Temperature: 21 ± 2 °C
 Humidity: 55 ± 15 %
 Air changes: At least 12 change/hour
 12-hour light/dark cycle

Study design and methods

In life dates: 1995-10-25 to 1996-04-04

Animal assignment and treatment

Two male and two female non-fasted rats were administered with a single oral dose of 40 mg [^{14}C]-glyphosate acid/kg by gavage. 24 and 48 hours after dosing, a heterosexual pair was killed and a qualitative whole body autoradiogram was performed. In addition, radioactivity was measured in urine, faeces and exhaled air.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) high performance liquid chromatography (HPLC).

Collection of excreta

Urine only was collected 6 hours after dosing, and separately together with faeces 12, 24, 36 and 48 hours after dosing from all surviving animals.

Urine collections comprised rinsing of each cage at each time point together with a thorough washing at the end of the study.

Faecal samples were analysed by sample oxidation followed by liquid scintillation counting whereas samples of urine and cage washings were analysed without intermediate processing.

Collection of exhaled air

The exhaled air from one heterosexual pair was passed through sodium hydroxide to trap any radioactivity expired as [^{14}C]-carbon dioxide. Subsamples of the contents of each trap were removed for radiochemical analysis at 6, 12 and 24 hours after dosing and were taken, without further processing, for liquid scintillation counting.

Whole body autoradiography

Immediately after scheduled sacrifice, each carcass was frozen rapidly and embedded in blocks of 2 % (w/v) aqueous carboxymethylcellulose. Longitudinal sagittal sections, 30 μm thick, were taken, mounted on adhesive tape and freeze-dried for approximately 48 hours. Autoradiograms were prepared by contact with autoradiographic film and exposed for periods of 2, 4 or 6 weeks.

Measurement of radioactivity - Liquid scintillation counting

Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.

Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel (60F₂₅₄) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15:5 v/v/v/v).

Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.

High performance liquid chromatography (HPLC)

To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mMol aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min. Radioactivity was detected using an on-line flow detector (liquid cell) and with UV absorption at 230 nm.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample, which was twice the liquid scintillation counter's background rate. The LOD for each carbon dioxide trap in this study was 0.01 % of the administered dose.

Results and discussion

Excretion of radioactivity

The results showed that 24 hours after dosing, excreted means of the administered dose in the urine and faeces amounted to 22.3 % and 55.5 % in males and 11.9 % and 83.8 % in females, respectively, whereas less than 0.2 % was excreted as carbon dioxide.

48 hours after dosing, excreted means of the administered dose in the urine and faeces increased to 34.0 % and 60.5 % in males and 12.5 % and 91.2 % in females, respectively.

The results of the excreted radioactivity in urine, faeces and exhaled air expressed as percentages of the administered radioactivity, together with the results for cage washings, are listed in Table B.6.1-20.

Table B.6.1-20: Excretion of radioactivity in urine, faeces, cage wash and expired air by male and female rats

	Time After Dosing (hours)	Males		Females	
		Rat 1	Rat 2	Rat 3	Rat 4
		(% of applied dose)		(% of applied dose)	
Urine	0-6	5.00	8.21	4.06	3.67
	6-12	5.30	8.67	4.32	4.12
	12-24	7.57	9.92	4.39	3.23
	24-36	N/A	4.30	N/A	0.93
	36-48	N/A	2.90	N/A	0.54
	Total	17.86	34.00	12.77	12.48
Faeces	0-12	30.62	0.21	54.27	32.63
	12-24	28.69	51.48	26.03	54.76
	24-36	N/A	1.65	N/A	2.71
	36-48	N/A	7.12	N/A	1.13
	Total	59.32	60.46	80.30	91.23
	Total	59.32	60.46	80.30	91.23
Exhaled ¹⁴ C- CO_2	0-6	0.07	N/A	0.08	N/A
	6-12	0.02	N/A	0.04	N/A
	12-24	0.02	N/A	0.03	N/A
	Total	0.11	N/A	0.14	N/A
Terminal Cage wash		1.12	0.98	1.43	0.41
Total		78.40	95.44	94.64	104.12

N/A not applicable

Whole body autoradiography

The whole body autoradiograms showed no marked differences in the distribution of radioactivity between male and female rats. The greatest intensity of labelling was present in the bone for both sexes, followed by the intestinal tract and the kidneys 24 hours after dosing with lesser to negligible amounts being present after 48 hours.

Conclusion by the Notifiers

Orally dosed glyphosate acid was excreted rapidly and predominantly in the faeces. 48 hours after dosing the greatest intensity of radiolabelling was in the bone and intestinal tract plus contents.

RMS comments

The study is considered supplementary despite its good quality. However, the number of animals of one per sex and timepoint is too low for definitive conclusions. All the parameters examined (i.e., excretion and distribution) had been addressed in other studies before. This previous knowledge was confirmed. Oral absorption was generally low but showed a remarkable interindividual variability.

B.6.1.2 Re-evaluation of previously known studies (mentioned in the 1998 DAR, ASB2010-10302) by the RMS

For the first EU evaluation of glyphosate, two separate “pairs” of ADME studies have been submitted which consisted of an in-life part, i.e., a complete ADME study according to OECD 417, and a subsequent metabolism part in which the metabolite pattern in urine and faeces was investigated.

- Monsanto studies
In life part: [REDACTED] (1988, TOX9552356)
Metabolism part: [REDACTED] (1988, TOX9552357)
- Feinchemie studies
In life part: [REDACTED] (1995, TOX9650071)
Metabolism (and organ/tissue distribution) part: [REDACTED] (1995, TOX9552251)

Furthermore, on behalf of Cheminova, [REDACTED] (1992, TOX9300343) performed a full-range ADME study in rats.

Re-evaluation by the RMS revealed that, although partly carried out before GLP became compulsory, all these studies mostly comply to modern standards and provide reliable information on kinetics and metabolism of glyphosate in the rat. In line with the original DAR, the studies by [REDACTED] (1988, TOX9552356), by [REDACTED] (1988, TOX9552357) and by [REDACTED] (1992, TOX9300343) are still regarded as fully acceptable by the RMS allowing comprehensive evaluation.

In contrast, quality rating of the studies by [REDACTED] (1995, TOX9650071) and [REDACTED] (1995, TOX9552251) has been declined to “supplementary”. This is due to the fact that radioactive residues in the organs, tissues and carcass were only investigated in one group receiving a low intravenous dose and, thus, do not reflect real exposure conditions. Even for this group that was treated by a highly artificial route, radioactivity in bone was not

determined. Furthermore, search for metabolites was confined to urine samples taken from high dose animals and possible abundance of faecal metabolites was not considered. Surprisingly, there was a different concentration given for the low oral dose by [REDACTED] (0.2 mg/kg bw) and [REDACTED] (0.3 mg/kg bw).

In addition, it must be emphasised that this study [as well as those by [REDACTED] (1995, ASB2012-11379) and by [REDACTED] (1996, ASB2012-11380)] did not include a group of rats receiving multiple administrations. Thus, a possible different kinetic behaviour following repeated exposure would not have been elucidated and information from other sources must be taken into account to cover this endpoint.

It could be argued that [REDACTED] (1995, TOX9650071) had employed a rather low dose of only 0.2 mg/kg bw(d) but the use of this concentration might even better reflect real exposure conditions than higher “low” doses would do. The advantage of that many ADME studies with glyphosate is in covering a wide dose range.

A preliminary study by [REDACTED] (1992, TOX9552358) included determination of pharmacokinetic parameters in the blood and whole-body autoradiography at a low dose of 30 mg/kg bw and is, because of its limited focus, now considered supplementary, too.

Two more (and rather old) studies were considered supplementary during the previous evaluation yet:

A study by [REDACTED] (1973, TOX9552355) is unique in that way that it was the only toxicokinetic experiment in which glyphosate was administered (for two weeks) to rats via the diet. Indeed, the dietary dose levels of 1 to 100 ppm appear rather low but these concentrations might reflect an actual residue concentration.

A second study by the same authors ([REDACTED] 1973, TOX9552353) is about toxicokinetics of glyphosate in male rabbits following single oral (gavage) administration of low doses in the range of 5.7 – 8.8 mg/kg bw. The results indicate similarities and differences between species such as a lower urinary clearance rate and higher tissue retention in rabbits as compared to the rat.

Although no batch number and no purity of the applied glyphosate was given and, therefore, in principle, assessment of these studies should have been turned into “unacceptable” now, the RMS suggests to keep them available as sources of useful additional information.

In contrast, there was another study also considered “supplementary” in the original DAR for which this rating actually cannot be maintained:

The metabolism study by [REDACTED] (1990, TOX9551961) is considered not acceptable due to serious reporting deficiencies. Thus, no information on the test material (batch, purity) is given and the applied dose not mentioned. The application method is not clear since on one hand “gastric lavage” (gavage?) is stated as the route but on the next page it is said that “rats were fed with the radioactive material”. Description of animals and animal handling is very poor. No information on toxicokinetics was obtained but this was apparently beyond the scope of the study that was performed to find out whether glyphosate was metabolised or not.

Likewise, a brief information ([REDACTED] 1973, TOX9552354) concerning kinetics and (absent) metabolism of aminomethylphosphonic acid (AMPA) is very poor and not acceptable from a today’s regulatory view.

B.6.1.3 Published information

Toxicokinetics and metabolism of glyphosate were seldom subject to investigations of industry-independent researchers and, thus, experimental data in open literature is scarce. The following paragraphs were transferred from the original DAR (1998, ASB2010-10302) and slightly amended for purposes of the RAR:

██████████ (1991, TOX9551791) reported an absorption rate from the gastrointestinal tract of 35 - 40 % of the total dose following the single oral administration of 10 mg of a mixture of ^{12}C - and ^{14}C -glyphosate per kg bw to male Sprague-Dawley rats. Urine and faeces were considered equally important routes of elimination. 7 days after application, total body burden was approximately 1 % of the administered dose and was primarily associated with the bone. Two hours following a single dose of the mixed test material, traces of a minor metabolite (<0.1 % of the dose applied) were detected beside the predominating parent in the colon tissue. This compound was also found in the gastrointestinal tract content of one rat at 28 hours post dosing and was considered likely to be aminomethyl phosphonic acid (AMPA) although the retention time for this metabolite was not identical to that for AMPA. The authors reported AMPA to be a product of metabolic activity of intestinal microbes.

██████████ (1992, TOX9551954) investigated (with contributions of ██████████ who are mentioned above) the elimination and tissue distribution of ^{14}C -glyphosate in male F344/N rats following oral and intravenous administration. After single low (5.6 mg/kg bw) or high (56 mg/kg bw) oral doses, more than 90 % of the applied radioactivity was eliminated within 72 hours. During the first 24 hours, approximately 50 % had been excreted in the faeces and nearly 30 % via the urine. It was assumed that the urinary radioactivity represented the amount of glyphosate absorbed. The peak blood levels occurred at 1 (low dose) or 2 (high dose) hours after dosing. Following an i.v. dose of 5.6 mg/kg bw, 90 % of the radioactivity was excreted in urine within the first 6 hours already. Glyphosate did not accumulate in the body. In a further group of rats receiving 5.6 mg/kg bw by oral gavage, only 1 % of the dose remained in the tissues after 24 hour. It is also stated that pretreatment with Roundup® via drinking water did not change the elimination pattern of glyphosate.

In a more recent paper that was included in the GTF dossier submitted for current evaluation, ██████████ (2009, ASB2012-11542) reported some parts of toxicokinetics of glyphosate (obtained from SIGMA CHEMICALS) in rats after single intravenous (i.v.) administration of 100 mg/kg bw or a single oral dose of 400 mg/kg bw. The focus was on plasma characteristics and distribution to the different compartments: "Serial blood samples were obtained after i.v. and oral administration. Plasma concentrations of glyphosate and its metabolite aminomethyl phosphonic acid (AMPA) were determined by HPLC method. After i.v. and oral administration, plasma concentration-time curves were best described by a two-compartment open model. For glyphosate, the elimination half-lives ($T_{1/2\beta}$) from plasma were 9.99 h after i.v. and 14.38 h after oral administration. The total plasma clearance was not influenced by dose concentration or route and reached a value of $0.995 \text{ l h}^{-1} \text{ kg}^{-1}$. After i.v. administration, the apparent volume of distribution in the second compartment (V_2) and volume of distribution at steady state (V_{ss}) were 2.39 and 2.99 l kg^{-1} , respectively, suggesting a considerable diffusion of the herbicide into tissues. After oral administration, glyphosate was partially and slowly absorbed with a T_{\max} of 5.16 h. The oral bioavailability of glyphosate was found to be 23.21 %. Glyphosate was converted to AMPA. The metabolite AMPA represented 6.49 % of the parent drug plasma concentrations. The maximum plasma concentrations of glyphosate and AMPA were 4.62 and $0.416 \mu\text{mol l}^{-1}$, respectively. The

maximum plasma concentration of AMPA was achieved at 2.42 h. For AMPA, the elimination half-life ($T_{1/2\beta}$) was 15.08 h after oral administration of glyphosate parent compound (*quoted from original article*)."

The RMS is not aware of any further scientific publications dealing with toxicokinetics and metabolism of glyphosate in laboratory animals. However, interesting additional information on urinary excretion of glyphosate in humans was provided that, however, did not alter the conclusions that were drawn from the many studies described above:

Data on urinary excretion in man following systemic exposure (presumed to be mainly dermal and inhalative) were obtained by [REDACTED] (2004, ASB2012-11528) as part of the "Farm Family Exposure Study". In this study, urinary glyphosate concentrations obtained from 48 farmers, their spouses, and their 79 children (4–18 years of age) were measured and evaluated. 24-hr composite urine samples were collected for each family member the day before, the day of, and for 3 days after a proven occupational glyphosate application. Sixty percent of the farmers had detectable levels of glyphosate in their urine on the day of application. The geometric mean (GM) concentration was 3 ppb, the maximum value was 233 ppb, and the highest estimated systemic dose (based on the latter measurement) was 0.004 mg/kg bw. Farmers who did not use rubber gloves had higher GM urinary concentrations than found in the other men (10 ppb vs. 2.0 ppb). Among spouses, only 4 % had detectable levels in their urine on the day of application. The maximum value was 3 ppb. In children, 12 % had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 ppb. All but one of the children with detectable concentrations had helped with the application or were present during herbicide mixing, loading, or application. This data is considered important because it is the only one that might give an idea about urinary excretion of glyphosate in humans following occupational exposure although [REDACTED] (2006, ASB2012-11888) had claimed some methodological deficiencies with regard to urine collection and absent correction for prior glyphosate exposure.

More recently, in 2013, a biomonitoring study was performed on behalf of the NGO "Friends of the earth" and its German partner organisation BUND by [REDACTED] [REDACTED] [REDACTED] and submitted to the RMS (ASB2013-8037). To our knowledge, this data has not been published in a scientific journal so far but is available in the internet. 182 urine samples from 18 European (EU and non-EU) countries (6 – 12 per country but mostly 10) were examined for glyphosate and AMPA by means of a modern analytical method (transformation of both compounds to two different derivatives followed by GC-MS/MS). This method appears very selective but it is not known whether it has been sufficiently validated so far. The LOQ for both, glyphosate and AMPA, was 0.15 µg/L. Creatinine was also measured as an internal proof for validity of the urine measurements.

For glyphosate, nearly one half (80) and for AMPA, more than one third (65) of the participants had urine concentrations above the LOQ. Maximum values of 1.82, 1.64 or 1.55 µg/L for glyphosate were found in samples obtained from Latvia, the UK, and Malta, respectively, but the mean value of 0.21 µg/L was much lower. (For calculation, the study author had included the samples with values below the LOQ and assumed a concentration of 0.075 µg/L, i.e., half the LOQ.) For AMPA, the maximum values of 2.63, 1.26, and 0.89 µg/L were measured in samples from Croatia, Belgium, and Malta with a mean urinary concentration of 0.18 µg/L for all involved people. It was surprising that in more than 30 cases the AMPA concentrations were higher than those of glyphosate, sometimes by 10 times

or more. In a few samples, AMPA values were rather high with glyphosate concentrations below the LOQ. These findings may arise doubts on the source of AMPA in urine because virtually no metabolism of glyphosate to AMPA may be expected.

The measured values themselves are considered reliable by the RMS. The results suggest that there is a certain exposure of European population to glyphosate, mainly by dietary intake. This is not surprising since glyphosate is a widely used active substance worldwide. Residues in food and feed may occur and are allowed if below the MRLs. Systemically available glyphosate (*i.e.*, the rather low percentage that is absorbed from the GIT) is excreted via the urine, virtually unchanged. Apparently, there is also some exposure to AMPA although its origin is less clear. However, due to the limited number of involved participants and the absence of any information about them (such as age, gender, body weight, social background, origin from urban or rural environments, nutrition habits) and the way how they were recruited, the study was only explorative and cannot be regarded as representative. The mean dietary exposure level cannot be estimated on this basis, neither for a single country nor for Europe in its whole. Moreover, no conclusion can be drawn to which extent the apparent differences in urinary levels of glyphosate in samples obtained in the different countries might reflect the actual use of glyphosate. (It was reported, *e.g.* that 8 out of 10 samples from Austria and 10 out of 12 from Switzerland were below the LOQ in contrast to only 3 of 10 from the UK or even 1 of 10 from Malta.)

In any case, none of the measured concentrations was of health concern since the exposure that may be calculated on this basis is far below the ADI. For an adult with 70 kg body weight, the newly proposed ADI of 0.5 mg/kg bw (see B.6.10 and Volume 1), would mean that the total daily intake of glyphosate might be as high as 35 mg. If 20 % is assumed to be orally absorbable (see above), up to 7 mg might be eliminated via the urine. Since the average urine volume is 1.5 – 2 L, theoretical urine concentrations of glyphosate in the magnitude of 3.5 to 4.7 mg/L would result. Even the maximum values measured by [REDACTED] (2013, ASB2013-8037) are less than 0.1 % of these expected concentrations proving a very low systemic dose that was received by the participants, presumably via the dietary route. With regard to AMPA, it must be emphasised that the glyphosate ADI also covers this metabolite and that the assessment does not change even when the low AMPA concentrations and those of glyphosate would be summed up.

It is interesting to note that the mean value of *ca* 0.2 µg/L was by five times lower than the geometric mean of glyphosate concentrations that were measured in a study in U.S. farmers on the third day following application, *i.e.*, with mainly dermal and inhalative exposure to be assumed. However, the maximum value on that post-application day 3 was 68 µg/L and on the day of glyphosate spraying even 233 µg/L ([REDACTED] 2004, ASB2012-11528). This comparison suggests that exposure of operators will normally exceed that of consumers and that, if operators are not on risk, dietary exposure should not be a matter of concern.

B.6.1.4 Data obtained with formulations

Not relevant for this section dealing with toxicokinetic behaviour and metabolism of the active substance. For dermal absorption, see B.6.12.

B.6.2 Acute toxicity including irritancy and skin sensitisation (Annex II A 5.2)

Introduction to this chapter by RMS:

The acute studies already evaluated in 2001 are only summarised in the tables below. These studies were not re-evaluated for the present renewal procedure. Even if some of these old studies would be considered now as not acceptable according to current standards, the assessment of the acute toxicity potential (incl. irritancy & skin sensitisation) of glyphosate will remain unchanged due to the huge amount of valid (new) studies. For details regarding studies reviewed during the 2001 EU evaluation please refer to the DAR.

The new submitted studies are summarized as well, additionally described in detail and commented by the RMS.

For higher efficiency of the review and for the sake of transparency, the descriptions of methods and study results in the GTF dossier were virtually not amended and even the conclusions were kept as provided. However, each study that is described in detail was commented by RMS. These remarks on bottom of each study description are clearly distinguished from the original submission by a caption and are always written in italics. In addition, the so-called "executive summaries" have been deleted to clearly represented the new studies.

B.6.2.1 Acute oral toxicity

Glyphosate acid and its salts have been extensively tested for acute toxicity, skin and eye irritation, and skin sensitisation. An amount of 145 acute studies was submitted either for the previous EU evaluation or in the present GTF dossier.

For the previous EU evaluation, a large number of oral toxicity studies in rats and mice were submitted that had been conducted with either glyphosate acid or its salts. In the current GTF dossier, a variety of additional studies in rats with administration of glyphosate acid and two more studies in rats and mice with the IPA salt were provided.

Table B.6.2-1: Summary of acute oral toxicity studies with glyphosate acid

Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
Studies from the 2001 evaluation Annex B-5.2.1.1.1, Glyphosate Monograph [REDACTED] 1995 (Sanachem) TOX9650909	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	> 2000 (limit test)	Slightly congested lungs, spleno-megaly, Liver: centrilobular congestion
Annex B-5.2.1.1.1, Glyphosate Monograph [REDACTED] 1994 (Herbex) TOX9500245	Rat, Sprague Dawley	1/sex/2000 ^{##} 5/sex/2000	95	Arachis oil	> 2000 (limit test)	No findings

Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
Annex B- 5.2.1.1.1, Glyphosate Monograph [REDACTED] 1994 (Alkaloida) TOX9650142	Rat, Wistar	5/sex/0 5/sex/5000	97.2	water	> 5000 (limit test)	♂: heart weights ↓
Annex B- 5.2.1.1.1, Glyphosate Monograph [REDACTED] 1995 (SIN) 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
Annex B- 5.2.1.1.1, Glyphosate Monograph [REDACTED], 1992 (Barclay) TOX9551810	Rat, Sprague Dawley	1/sex/2000 ^{##} 5/sex/2000	>97	water	> 2000 (limit test)	No findings
Annex B- 5.2.1.1.1, Glyphosate Monograph [REDACTED] 1991 (FSG) 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
Studies from the 2001 evaluation	Annex B- 5.2.1.1.1, Glyphosate Monograph [REDACTED] 1990 (AGC) 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
	Annex B- 5.2.1.1.1, Glyphosate Monograph [REDACTED] 1989 (CHE) TOX9552319	Rat, Sprague Dawley 5/sex/5000	98.6	0.5 % CMC	> 5000 (limit test)	Piloerection, reduced activity, ataxia (♂ only)
Studies not reviewed in the 2001 evaluation	IIA 5.2.1/01 [REDACTED] 2009 (HAG) ASB2012- 11381	Rat, Sprague Dawley 5/females/5000	96.4	water	> 5000 (limit test)	decreased activity, diarrhoea, piloerection, polyuria, salivation

Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
IIA 5.2.1/02 [REDACTED] 1995 (ALS) ASB2012- 11382	Rat, Sprague Dawley	5/sex/5000	95.68	0.5 % CMC	> 5000 (limit test)	decreased spontaneous motor activity and salivation
IIA 5.2.1/03 [REDACTED] 1995 ASB2012- 11383	Mice, ICR	5/sex/5000	95.68	0.5 % CMC	> 5000 (limit test)	decreased spontaneous motor activity, sedation and crouching position
IIA 5.2.1/04 [REDACTED] 2009 (EXC) ASB2012- 11384	Rat, Wistar	3 females/2000 (step 1) 3 females/2000 (step 2)	96.66	water	> 2000	No findings
IIA 5.2.1/05 [REDACTED], 2009 (HAG) 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
IIA 5.2.1/06 [REDACTED] 2010 (HAG) 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
IIA 5.2.1/07 [REDACTED] 2010 (HAG) 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
IIA 5.2.1/08 [REDACTED] 2005 (HAG) ASB2012- 11388	Rat, Sprague- Dawley	3 females/5000	97.23	water	> 5000 (limit test)	Diarrhea, ano- genital & facial staining, reduced faecal volume
IIA 5.2.1/09 [REDACTED] 2008 (HAG) ASB2012- 11389	Rat, Wistar	3 females/2000 (step 1) 3 females/2000 (step 2)	98.05	water	> 2000 (limit test)	No findings
IIA 5.2.1/10 [REDACTED] 2007 (NUF) ASB2012- 11390	Rat, HanRcc: WIST	2 x 3 ♀/2000	95.1	PEG 300	> 2000 (limit test)	Slightly ruffled fur
IIA 5.2.1/11 [REDACTED], 1988 (MON) Z35389	Rat, Sprague Dawley	5/sex/5000	97.76	water	> 5000	diarrhea, apparent urinary incontinence and hair loss on the abdomen

Studies not reviewed in the 2001 evaluation

Studies not
reviewed in
the 2001
evaluation

WARNING: This document is a draft and should not be read in isolation. Registration must not be granted on the basis of this document.

Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
IIA 5.2.1/12 [REDACTED] 1979 (MON) Z35541	Rat, Wistar	5/sex/2500 5/sex/3500 5/sex/5000 5/sex/7000 5/sex/9900	99	water	> 5000	Mortality: 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
IIA 5.2.1/13 [REDACTED] 1996 (SYN) TOX2000- 1982	Rat	5/sex/5000	95.6	water	> 5000	No findings
IIA 5.2.1/14 [REDACTED] 2007 (SYN) ASB2012- 11391	Rat	3 ♀/5000	96.1	Water	> 5000	Ruffled fur, hunched posture
IIA 5.2.1/15 [REDACTED], 2011 (SYN) ASB2012- 11392	Rat	3 ♀/5000	96.3	0.5 % CMC	>5000	No findings
Studies from the 2001 evaluation	Annex B- 5.2.1.1.2, Glyphosate Monograph [REDACTED], 1991 (FSG) TOX9551089	Mice, 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
	Annex B- 5.2.1.1.2, Glyphosate Monograph [REDACTED] (I.Pi. Ci) TOX9551624	Mice, Charles River 5/sex/2000	technical	0.5 % CMC	> 2000 (limit test)	Piloerection, hunched posture, hypoactivity

Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
Annex B- 5.2.1.1.2, Glyphosate Monograph [REDACTED] 1991 (CHE)TOX95 52320	Mice, Bom:NM RI	5/sex/2000	98.6	water	> 2000 (limit test)	Piloerection, sedation

CMC = carboxymethylcellulose

Table B.6.2-2: Summary of acute oral toxicity studies with glyphosate salts

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Salt type Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
Studies from the 2001 evaluation	Annex B- 5.2.1.2.1, Glyphosate Monograph [REDACTED], 1995 (Sanachem) TOX9650910	Rat, Sprague Dawley	5/sex/2000	IPA 61.8	none	> 2000 (limit test)	severely congested lungs, splenomegaly, hepatomegaly with centrilo- bular conges- tion, subcap- suloar renal petechiae
	Annex B- 5.2.1.2.1, Glyphosate Monograph [REDACTED] 1994 (MON / CHE) TOX9552322	Rat, Sprague Dawley	5/sex/2000	IPA 62.2	none	> 5000 (limit test)	No findings
	Annex B- 5.2.1.2.1, Glyphosate Monograph [REDACTED] 1989 (I.Pi.Ci) TOX9551623	Rat, Wistar	5/sex/2000	IPA 62% in water	none	> 2000 (limit test)	No findings
Studies from the 2001 evaluation	Annex B- 5.2.1.2.1, Glyphosate Monograph [REDACTED], 1981 (MON / CHE) TOX9552321	Rat, Sprague- Dawley	5/sex/5000	IPA 65	none	> 5000 (limit test)	Pale colored kidneys and hydronephrosis in few ♀

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Salt type Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
	Annex B- 5.2.1.2.2, Glyphosate Monograph [REDACTED] 1987 (SIN) TOX9500376	Mice, ICR	5/sex/0 (PEG) 5/sex/3125 5/sex/3625 5/sex/4125 5/sex/4625 5/sex/5125	IPA 41	PEG 200	3669 (confidence interval: 3489 – 3858)	Dose-related mortality in all IPA-treated groups; immobility, tremor, hyperemia of the ears
Study from the 2001 evaluation	Annex B- 5.2.1.2.2, Glyphosate Monograph [REDACTED] 1987 (SIN) TOX9500375	Mice, ICR	5/sex/0 (PEG) 5/sex/3125 5/sex/3625 5/sex/4125 5/sex/4625 5/sex/5125	IPA 64	PEG 200	4373 (confidence interval: 4144 – 4644)	Dose-related mortality in all IPA-treated groups; immobility, tremor, hyperemia of the ears
Studies not reviewed in the 2001 evaluation	IIA 5.2.1/16 [REDACTED], 1995 (MON) ASB2012- 11393	Mice CD-1	5/sex/5000	IPA 62.34	none	> 5000	Slight reduction in body weight gain in males at 5000 mg/kg/day versus controls
	IIA 5.2.1/17 [REDACTED], 1999 (NUF) ASB2012- 11394	Rat, Sprague Dawley	5/sex/5000	IPA 62%	none	> 5000 (limit test)	♀: anogenital staining, soft faeces or diarrhoea
Study from the 2001 evaluation	Annex B- 5.2.1.2.1, Glyphosate Monograph [REDACTED] 1987 (MON / CHE) Z85869 & TOX9552323	Rat, Sprague- Dawley	1/sex/2000 5/sex/5000 5/sex/7500	NH ₄ - salt 90.8	none	4613 (confidence interval: 3511 – 5716)	5000 and 7500 mg/kg bw: mortality; ataxia, decreased activity, diarrhea, labored breathing, color changes of lungs (dark red), GI tract of dead rats with dark/red fluid

Tier II summaries are presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.

Reference: IIA, 5.2.1/01
Report: [REDACTED] (2009) Glyphosate: Acute Oral Toxicity Study (UDP) In Rats.
[REDACTED]
Data owner: Helm AG
Report No.: 12170-08
Date: 2009-03-11, not published
ASB2012-11381
Guidelines: US EPA OPPTS 870.1100
Equivalent to OECD 425 (2008).
Deviations: Humidity was in the range of 33-89 % instead of 30-70 %. This deviation did not affect the study outcome
GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate
Identification: Glyphosate Tech Grade Mixed 5-Batch
Description: White powder
Lot/Batch #: 080704-1 thru 5
Purity: 96.40 %
Stability of test compound: No data given in the report.
Vehicle and/or positive control: Deionised water
Test animals:
Species: Rat albino
Strain: Sprague-Dawley
Source: [REDACTED]
Age: 7 - 8 weeks
Sex: Female
Weight at dosing: 160 - 187 g
Acclimation period: 5 days
Diet/Food: Formulab #5008 (PMI Feeds Inc.), *ad libitum* except for approx. 16 h before dosing
Water: Tap water, *ad libitum*
Housing: Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30 - 89 %
Air changes: 10 - 12/hour
12-hour light/dark cycle

Study design and methods

In life dates: 2008-11-11 to 2008-11-27

Animal assignment and treatment:

A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The dosing volume was 12.5 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.

On Day 14 after dosing, each animal was euthanised by an overdose of CO₂. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Clinical signs in one animal included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident at Day 8.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (glyphosate) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and the evaluation is agreed.

Reference: IIA, 5.2.1/02

Report: [REDACTED] (1995) HR-001: Acute Oral Toxicity Study In Rats.

Data owner: Arysta Life Sciences

Report No.: IET 94-0134

Date: 1995-02-20, not published

ASB2012-11382

Guidelines: OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)

Deviations: None

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material:

Identification: Glyphosate technical, Code: HR-001

Description: White crystal

Lot/Batch #:	940908-1
Purity:	95.68%
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	0.5% carboxymethyl-cellulose (CMC)
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crj:CD), SPF
Source:	
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	♂ 168 - 179 g; ♀ 125 - 142 g
Acclimation period:	7 days
Diet/Food:	Pellet Diet MF (Oriental Yeast Co., Japan), <i>ad libitum</i> except for an overnight fast before dosing and about 3 h after dosing
Water:	Tap water, <i>ad libitum</i>
Housing:	Wire-mesh stainless steel cages in groups of 5 animals/sex/cage.
Environmental conditions:	Temperature: 23 ± 3°C Humidity: 55 ± 15% Air changes: 12/hour 12-hour light/dark cycle

Study design and methods

In life dates: 1995-01-24 to 1995-02-07

Animal assignment and treatment:

A group of five fasted rats per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anesthesia and subjected to gross necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Decreased spontaneous motor activity was observed in five males and three females as well as salivation in one male. These signs were observed at 1 and 3 hours after the administration.

Body weight: No body weight losses were recorded on Day 7 and 14 after administration when compared with the body weights on the day of administration.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (HR-001) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, HR-001 is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and the evaluation is agreed.

Reference:

IIA, 5.2.1/03

Report:

(1995) HR-001: Acute Oral Toxicity Study In Mice.

Data owner: Arysta Life Sciences

Report No.: IET 94-0133

Date: 1995-02-20, not published

ASB2012-11383

Guidelines:

OECD 401 (1987), JMAFF 59 Noh San 4200 (1995), US EPA (1984)

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Identification:

Glyphosate technical, Code: HR-001

Description:

White crystal

Lot/Batch #:

940908-1

Purity:

95.68 %

Stability of test compound:

No data given in the report.

Vehicle and/or positive control:

0.5 % carboxymethyl-cellulose (CMC)

Test animals:

Species:

Mice

Strain:

ICR (Crj:CD-1), SPF

Source:

Age:

6 weeks

Sex:

Males and females

Weight at dosing:

♂ 29.4 - 32.7 g; ♀ 22.8 - 25.8 g

Acclimation period:

7 days

Diet/Food:

Pellet Diet MF (Oriental Yeast Co., Japan), *ad libitum* except for approx. 2 h before dosing, and 3 h after dosing

Water:

Tap water, *ad libitum*

Housing:

Aluminium cages with wire-mesh floors in groups of 5 animals/sex/cage.

Environmental conditions: Temperature: 23 ± 3 °C
 Humidity: 55 ± 15 %
 Air changes: 12/hour
 12-hour light/dark cycle

Study design and methods

In life dates: 1995-01-24 to 1995-02-07

Animal assignment and treatment:

A group of five fasted mice per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anesthesia and subjected to gross necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Decreased spontaneous motor activity was observed in one male and one female as well as sedation and crouching position in another male. These signs were observed at 1 and 3 hours after the administration.

Body weight: 7 days after administration, a slight body weight loss (0.5 g) was observed in one male when compared with the body weight on the day of administration. No body weight losses were recorded in any animal 14 days after the administration.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (HR-001) in mice was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, HR-001 is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and the oral LD₅₀ of > 5000 mg/kg bw in mice is agreed.

Reference: IIA, 5.2.1/04

Report: [REDACTED] (2009) Glyphosate Technical: Acute Oral Toxicity Study in Rats.

[REDACTED]
Data owner: Excel

Report No.: C22864

Date: 2009-04-02, not published

ASB2012-11384

Guidelines: OECD 423 (2001)

Commission Regulation (EC) No 440/2008 (2008), method B.1 tris

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:

Identification: Glyphosate Technical

Description: Solid

Lot/Batch #: GI-1045

Purity: 96.66 %

Stability of test compound: (Stable under storage conditions.)
Expiry date: July 2010.

Vehicle and/
or positive control: Purified water

Test animals:

Species: Rat

Strain: HanRcc: WIST (SPF)

Source:

Age: 11 weeks

Sex: Female

Weight at dosing: 181.0 – 198.7 g

Acclimation period: 7 days

Diet/Food: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 61/08 (Provimi Kliba AG, 4303 Kaiseaugst, Switzerland) ad libitum (except for the overnight fasting period prior to intubation and approximately 3-4 hours post dose).

Water: Tap water, *ad libitum*

Housing: In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Shill AG, 4132 Muttensz / Switzerland).

Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30 - 70 %
Air changes: 10 - 15/hour
12-hour light/dark cycle

Study design and methods

In life dates: 2009-02-13 to 2009-02-03 and 2009-02-05

Animal assignment and treatment:

Two groups of three fasted females each received the test material at a dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical signs of toxicity were made at least five times on the day of dosing (Day 1) and at twice daily thereafter during days 2-15. Individual body weights were recorded just prior to dosing and on Days 8 and 15.

On Day 15 after dosing, each animal was euthanised by CO₂ asphyxiation. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion

Mortality: No deaths occurred during the study.

Clinical observations: No clinical signs were observed during the course of the study.

Body weight: The body weight of the animals was within the range commonly recorded for this strain and age.

Necropsy: No macroscopic findings were recorded at necropsy.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate technical is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and the estimated oral LD₅₀ > 2000 mg/kg bw is agreed.

Reference: IIA, 5.2.1/05

Report: [REDACTED] (2009) Acute Oral Toxicity Study of Glyphosate TC in Rats.

Data owner: Helm AG

Report No.: 23910

Date: 2009-06-16, not published

ASB2012-11385

Guidelines: EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.

Deviations: A personnel change in the Head of the Quality Assurance Unit did not affect the validity of the study outcome.

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate TC

Identification: Glyphosate technical grade

Description: White powder

Lot/Batch #: 20080801

Purity: 98.8 %

Stability of test compound: 2010-08-01

Vehicle and/or positive control: 0.8 % aqueous hydroxypropylmethylcellulose gel

Test animals:

Species: Rat albino

Strain / Stock: CD / CrI:CD(SD)

Source: [REDACTED]

Age: 50 - 51 days

Sex: Female

Weight at dosing:	167 - 186 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), <i>ad libitum</i> except for approx. 16 h before dosing
Water:	Tap water, <i>ad libitum</i>
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % 12-hour light/dark cycle

Study design and methods:

In life dates: 2009-02-04 to 2009-03-04

Animal assignment and treatment:

A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6, and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.

On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No clinical signs were observed during the study.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (Glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and evaluation is agreed, the oral LD₅₀ is greater than 2000 mg/kg bw.

Reference: IIA, 5.2.1/06
Report: [REDACTED] (2010) Acute Oral Toxicity Study of Glyphosate TC in Rats.
[REDACTED]
Data owner: Helm AG
Report No.: 24874
Date: 2010-01-06, not published
ASB201211386
Guidelines: EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.
Deviations: There were no deviations from the study plan.
GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate TC
Identification: Glyphosate technical grade
Description: White powder
Lot/Batch #: 2009051501
Purity: 96.4 %
Stability of test compound: 2011-05-15
Vehicle and/or positive control: 0.8 % aqueous hydroxypropylmethylcellulose
Test animals:
Species: Rat albino
Strain / Stock: CD-1 CrI:CD(SD)
Source: [REDACTED]
Age: Approx. 7 weeks
Sex: Female
Weight at dosing: 171 - 192 g
Acclimation period: 5 days
Diet/Food: ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water: Tap water, *ad libitum*
Housing: Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions: Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 %
12-hour light/dark cycle

Study design and methods:

In life dates: 2010-10-15 to 2010-11-10

Animal assignment and treatment:

A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.

On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No clinical signs were observed during the study.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (Glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and the oral LD₅₀ > 2000 mg/kg bw is agreed.

Reference:

IIA, 5.2.1/07

Report:

[REDACTED] (2010)

Acute Oral Toxicity Study of Glyphosate TC in Rats.

Data owner: Helm AG

Report No.: 24602

Date: 2010-02-19, not published

ASB2012-11387

Guidelines:

EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.

Deviations:

There were no deviations from the study plan.

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Glyphosate TC

Identification:

Glyphosate technical grade

Description:

White powder

Lot/Batch #:

20090506

Purity:

97.3 %

Stability of test compound: May 2011

Vehicle and/or positive control: 0.8 % aqueous hydroxypropylmethylcellulose

Test animals:

Species: Rat albino

Strain / Stock: CD / CrI:CD(SD)

Source: [REDACTED]

Age: Approx. 7 - 8 weeks

Sex: Female

Weight at dosing: 154 - 196 g

Acclimation period: 5 days

Diet/Food: ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing

Water: Tap water, *ad libitum*

Housing: Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.

Environmental conditions: Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 %
12-hour light/dark cycle

Study design and methods:

In life dates: 2009-10-26 to 2009-11-24

Animal assignment and treatment:

A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6, and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.

On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No clinical signs were observed during the study.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (Glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and based on the results the oral LD₅₀ >2000 mg/kg bw/d is agreed.

Reference: IIA, 5.2.1/08
Report: [REDACTED] (2005)
Glyphosate Acid Technical – Acute Oral Toxicity Up and Down
Procedure in Rats.
[REDACTED]
Data owner: Helm AG
Report No.: 15274
Date: 2005-04-04, not published
ASB2012-11388
Guidelines: US EPA OPPTS 870.1100 (2002)
OECD 425 (2001).
Deviations: There were no deviations from the study plan.
GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate Acid Technical
Identification: Glyphosate Acid Technical
Description: White crystalline powder
Lot/Batch #: 040205
Purity: 97.23 %
Stability of test compound: Test substance was expected to be stable for the duration of testing.
Vehicle and/or positive control: Distilled water
Test animals:
Species: Rat albino
Strain: Sprague-Dawley derived
Source: [REDACTED]
Age: 11 weeks
Sex: Female
Weight at dosing: 222 - 235 g
Acclimation period: 21 or 23 days
Diet/Food: Purina Rodent Chow #5012, *ad libitum* except for overnight fasting before dosing
Water: Filtered tap water, *ad libitum*
Housing: Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions: Temperature: 19-23 °C
12-hour light/dark cycle

Study design and methods:

In life dates: 2004-05-04 to 2004-05-20

Animal assignment and treatment:

A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The test substance was administered as a 50 % w/w suspension in distilled water. Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14.

On Day 14 after dosing, each animal was euthanised by an overdose of CO₂. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Clinical signs noted for all animals included diarrhea, ano-genital and facial staining, and/or reduced fecal volume. All animals recovered by Day 4 and appeared active and healthy for the remainder of the 14-day observation period.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (glyphosate acid technical) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate Acid Technical is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and the evaluation is agreed.

Reference:

IIA, 5.21/09

Report:

(2008) Acute Oral Toxicity Study in

Data owner: Helm AG

Report No.: RF-3996.305.475.07

Date: 2008-09-16, not published

ASB2012-11389

Guidelines:

OECD guideline 423 (2001).

Deviations:

The experimental phase initiation and conclusion dates were updated. This deviation did not affect the study outcome.

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:	Glyphosate Technical
Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat albino
Strain:	Wistar Hannover
Source:	
Age:	8 - 9 weeks
Sex:	Female
Weight at dosing:	172 - 205 g
Acclimation period:	6 days
Diet/Food:	Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), <i>ad libitum</i> except for fasting overnight before dosing
Water:	Filtered drinking water, <i>ad libitum</i>
Housing:	Groups of three rats per cage were held in polypropylene rodents cages with wire mesh tops and bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: min. 10/hour 12-hour light/dark cycle

Study design and methods

In life dates: 2007-09-12 to 2008-06-11

Animal assignment and treatment:

A group of three fasted females received the test material at a dose level of 2000 mg/kg bw by oral gavage in a stepwise manner. Observations for mortality and clinical/behavioural signs of toxicity were made once within the first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were recorded just prior to dosing (Day 0) and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized by an overdose of CO₂. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No clinical signs of toxicity were observed in females treated with 2000 mg/kg bw.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The acute oral LD₅₀ cutoff-value of the test material (glyphosate technical) in female rats was estimated to be 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria Glyphosate Technical is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable. The limit dose of 2000 mg/kg bw (not 5000 mg/kg bw/d) did not cause signs of toxicity, the acute oral LD₅₀ is >2000 mg/kg bw.

Reference:

IIA, 5.2.1/10

Report:

[REDACTED] (2007)

GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study in rats

[REDACTED]
Data owner: Nufarm

Report No.: BO2272

Date: 2007-03-01, unpublished

ASB2012-11390

Guidelines:

Japanese guideline Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF),

Guidelines for Preparation of Study Results, Acute oral toxicity studies. Guideline 201-1

Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005.

English translation by ACIS on 17 Oct 2005.

Directive 2004/173/EC, 8.1 tris "Acute Oral Toxicity-Acute Toxic Class Method", April 29, 2004.

OECD Guidelines for the Testing of Chemicals, Number 423 "Acute Oral Toxicity - Acute Toxic Class Method", adopted 17 December 2001.

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Identification:

Glyphosate Technical (NUP 05068)

Description:

White powder

Lot/Batch #:

200609062

Purity:

95.1 %

Stability of test compound:

Stable under storage conditions.

Vehicle and/or positive control: Polyethylene glycol 300 (PEG 300)

Test animals:

Species: Rat

Strain: HanRcc:WIST (SPF)

Source: [REDACTED]

Age: 11 weeks

Sex: Female

Weight at dosing: 160 - 187 gg

Acclimation period: 5 days

Diet/Food: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/Switzerland) ad libitum.

Water: Tap water, ad libitum

Housing: In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Schill AG, CH-4132 Muttenz/Switzerland).

Environmental conditions: Temperature: $22 \pm 3^{\circ}\text{C}$
Humidity: 30 - 70 %
Air changes: 10 - 15/hour
12-hour light/dark cycle

Study design and methods:

In life dates: 2006-12-12 to 2007-01-04

Animal assignment and treatment:

The animals received a single dose of the test item by oral gavage administration at 2000 mg/kg body weight after being fasted for approximately 18 to 19 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing. The dosing volume was 10 mL/kg body weight. OBSERVATIONS for Mortality and Viability: Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.

Body weights: On test days 1 (prior to administration), 8 and 15.

Clinical signs: Daily during the acclimatisation period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Once daily during days 2-15. All abnormalities were recorded.

NECROPSY: All animals were killed at the end of the observation period by Carbon dioxide asphyxiation.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Clinical signs in one animal included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident at Day 8.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The median lethal dose of Glyphosate Technical (NUP 05068) after single oral administration to female rats, observed over a period of 14 days is: LD50 (female rat) > than 2000 mg/kg body weight.

Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate Technical (NUP 05068) is not to be classified for this end point.

Comment by RMS:

The study is considered acceptable and evaluation is agreed.

Reference:

IIA, 5.2.1/11

Report:

[REDACTED] (1988) Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats

Data owner: Monsanto

Monsanto Report No.: FD-88-29

Date: 1988-06-08, not published

Z35389

Guidelines:

US EPA 81-1

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Identification:

Glyphosate

Description:

White powder

Lot/Batch #:

XLI-55

Purity:

97.76 %

Stability of test compound:

No data given in the report.

Vehicle and/or positive control:

Distilled water

Test animals:

Species:

Rat

Strain:

Sprague-Dawley

Source:

Age:

Not specified

Sex:

Male and female

Weight at dosing:

♂ 300 - 332 g; ♀ 217 - 222 g

Acclimation period:

At least 5 days

Diet/Food: NIH Open Formula 07 Rat and Mouse Diet, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum* (except when fasted overnight prior to dosing)

Water: Tap water, *libitum*

Housing: Wire mesh cages

Environmental conditions: Temperature: 20 – 23.9 °C
Humidity: 40 – 70 %
Air changes: Not specified
Light cycle: 12 hour light/dark cycle

Study design and methods:

In life dates: 1988-04-05 to 1988-04-19

Animal assignment and treatment:

Groups of five male and five female rats received the test material at a dose level of 5000 mg/kg body weight by oral gavage as a 50 % w/v aqueous suspension. Observations for mortality and signs of toxicity were made three times on the day of dose administration and twice daily thereafter. Body weights were recorded prior dose administration on study day 1, and on days 8 and 15 (terminal sacrifice). A gross necropsy was performed on all animals at the time terminal sacrifice (day 15) and all abnormalities were recorded.

Results and discussion

Mortality: No mortalities occurred.

Clinical observations: Clinical signs included diarrhoea, apparent urinary incontinence, and hair loss on the abdomen.

Body weight: Body weight gain was noted for all animals.

Necropsy: No internal abnormalities were noted during gross necropsy examination of the animals.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (glyphosate) in rats was estimated to be 5000 mg/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate should not be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and evaluation is agreed.

Reference:

IIA, 5.2.1/12

Report:

[REDACTED] (1979) Acute Oral Toxicity Study In Rats.

[REDACTED]

Data owner: Monsanto

Monsanto Report No.: BND-77-428

Date: 1979-08-06, not published

Z35541

Guidelines:

None (pre-guideline)

Deviations:

Not specified

GLP:

no (pre-GLP)

Acceptability:

Please see comment by RMS

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Technical
Description:	Fine white powder
Lot/Batch #:	XHI-180
Purity:	99 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Rat
Strain:	Wistar
Source:	
Age:	Not specified
Sex:	Male and female
Weight at dosing:	225 - 294 g
Acclimation period:	Not specified
Diet/Food:	<i>ad libitum</i> (except when fasted for approximately 18 hours prior to dosing)
Water:	<i>ad libitum</i>
Housing:	Individually
Environmental conditions:	Temperature: Not specified Humidity: Not specified Air changes: Not specified Light cycle: Not specified

Study design and methods:

In life dates: Not specified

Animal assignment and treatment:

Groups of five male and five female rats received the test material at a dose levels of 2.5, 3.5, 5.0, 7.0, and 9.9 g/kg body weight by oral gavage. The test material was administered by oral intubation as a 25 % w/v solution in distilled water. Observations for mortality and overt signs of effect were made at 0-2 and 4-6 hours following dosing and twice daily thereafter (early morning and late afternoon) for fourteen days. Body weights were recorded prior to fasting, on Day 7, and on Day 14 of the study. A gross necropsy was performed on all animals at the time of death or terminal sacrifice (Day 14). All abnormalities were recorded.

Results and discussion

Mortality: Mortalities in the study are as indicated below in Table B.6.2-3.

Table B.6.2-3: Summary of Mortality

Dose Level (g/kg body weight)	Mortality/Total Number of Animals Dosed
2.5	1/10
3.5	1/10
5.0	3/10
7.0	8/10
9.9	10/10

Clinical observations: Clinical signs included 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.

Body weight: For the 2.5, 3.5, 5.0, and 7.0 g/kg body weight dose levels, although some animals lost weight between 7 and 14 days, all surviving animals gained weight throughout the study.

Necropsy: A summary of the gross necropsy findings for the decedents and the animals necropsied at the conclusion of the 14-day observation period is presented in Table B.6.2-4.

Table B.6.2-4: Summary of Necropsy Findings

Dose Level (g/kg body weight)	Animals Necropsied at 14 Days	Decedents
2.5	Discoloured lungs, liver, and/or kidneys	Urinary and faecal staining of the abdomen Discoloured lungs Fluid filled stomach Fluid filled and/or distended intestines
3.5	Discoloured lungs, liver, and/or kidneys Air filled intestines	Discoloured lungs
5.0	No observations	Oral and/or nasal discharge Urinary and/or faecal staining of the abdomen Discoloured lungs and/or liver Fluid filled and/or discoloured stomach and/or intestines
7.0	Discoloured lungs, liver, and/or kidneys Air filled intestines	Oral discharge Fluid filled intestines and/or stomach Discoloured liver, and/or kidneys Urinary and/or faecal staining of the abdomen
9.9	Not applicable	Discoloured lungs, liver, and/or kidneys Fluid filled intestines and/or stomach Oral and/or nasal discharge Urinary staining of the abdomen

Conclusion by the Notifiers

The oral LD₅₀ of the test material (glyphosate) in rats was estimated to be 5.6 g/kg body weight with 95 % confidence limits of 4.9 to 6.3 g/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for acute oral toxicity.

Comment by RMS:

This old study was conducted prior to GLP and not according to any Test Guideline, subsequently some reporting deficiencies were apparent. Therefore, this study is considered

to be supplementary. Under the conditions of the present study the oral LD₅₀ value of > 5000 mg/kg bw is agreed.

Reference: IIA, 5.2.1/13
Report: [REDACTED] (1996) Glyphosate Acid: Acute Oral Toxicity Study In Rats.
[REDACTED]
Data owner: Syngenta
Report No.: CTL/P/4660
Date: 1996-08-23, not published
TOX2000-1982
Guidelines: OECD 425 (2001)
US EPA OPPTS 870.1100 (2002)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS.

Materials and methods

Test material:
Identification: Glyphosate Acid
Description: Technical, white solid
Lot/Batch #: P24
Purity: 95.6 %
Stability of test compound: The test substance was used within the expiry date.
Vehicle and/
or positive control: Deionised water
Test animals:
Species: Rat
Strain: Alpk:AP_fSD (Wistar-derived)
Source: [REDACTED]
Age: Young adult
Sex: Male and female
Weight at dosing: 233-260 g (males), 197-225 g (females)
Acclimation period: At least 6 days
Diet/Food: Diet (PCD), supplied by Special Diet Services Limited, Witham, Essex, UK *ad libitum* (except overnight immediately prior to dosing).
Water: Mains water *ad libitum*
Housing: 5/cage, sexes separately in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study.

Environmental conditions: Temperature: 21 ± 2 °C
 Humidity: 40 – 70 %
 Air changes: Approximately 25-30/hour
 12-hour light/dark cycle

Study design and methods:

In life dates: 1995-03-16 to 1995-03-30

Animal assignment and treatment:

In an acute oral toxicity study, a group of five male and five female, fasted, young adult Alpk:AP_fSD (Wistar-derived) rats were given a single oral dose of 5000 mg/kg bw of glyphosate acid by gavage. The test substance was diluted in deionised water. The volume of the dose was calculated for each animal according to its weight at the time of dosing and a standard volume of 10 mL/kg of the dosing preparation was administered.

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic toxicity once within 2 hours of dosing and again between 4 and 7 hours after dosing. Subsequent observations were made daily, up to day 15.

The animals were weighed on the day before dosing (day -1), immediately before dosing (day 1) and on days 3, 8, 8 and 15.

All animals were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera.

Results and discussion

Mortality: There were no mortalities.

Clinical observations: There were no signs of systemic toxicity.

Body weight: All animals lost weight initially due to the pre-dose fast, but all had exceeded their initial weight by day 3, and apart from a transient weight loss in one female, continued to gain weight throughout the remainder of the study.

Necropsy: Red or mottled areas in the lung or red areas in the thymus were seen in three males and two females. These are common spontaneous findings in rats of this age and strain and are considered not to be treatment-related.

Conclusion by the Notifier

The oral LD₅₀ of the test material (glyphosate acid) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate acid is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and evaluation is agreed.

Reference

IIA, 5.2.1/14

Report:

██████████ (2007) Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure). ██████████

Data owner: Syngenta

Report No.: B02755

Date: 2007-02-08, not published

ASB2012-11391

Guidelines: OECD 425 (2001)
US EPA OPPTS 870.1100 (2002)
Japanese MAFF 12 NohSan No. 8147

Deviations: None

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material:
Identification: Glyphosate technical material
Description: Technical, white powder
Lot/Batch #: 0507
Purity: 96.1 %
Stability of test compound: Re-certification date August 2008. Stable under storage conditions (room temperature range 20 ± 5 °C, protected from light and humidity).
Vehicle and/or positive control: Purified water (deionised water processed and treated by the PURELAB Option-R unit which links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation).
Test animals:
Species: Rat
Strain: HanRcc:WIST (SPF)
Source: [REDACTED]
Age: 11 weeks
Sex: Female
Weight at dosing: 183.0-188.9 g
Acclimation period: 5-7 days
Diet/Food: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) *ad libitum* (except for pre-dose fast).
Water: Community tap water *ad libitum*
Housing: Individually in Makrolon type-3 cages with standard softwood bedding
Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30 - 70 %
Air changes: 10 - 15/hour
12-hour light/dark cycle

Study design and methods:

In life dates: 2006-12-06 to 2006-12-26

Animal assignment and treatment:

In an acute oral toxicity study, a group of three, fasted, 11 week old, HanRcc:WIST (SPF) female rats was given a single oral dose of glyphosate technical material (96.1 % w/w glyphosate acid) at a concentration of 5000 mg/kg body weight by gavage. The test substance was diluted in vehicle (purified water) and dosed at a volume dosage of 10 mL/kg body weight.

Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. The first animal was treated at a dose level of 5000 mg/kg body weight. As no mortality or significant clinical signs were observed, two additional animals were sequentially dosed at 5000 mg/kg such that a total of 3 animals were tested. No mortalities were observed, therefore the study was terminated.

The animals were examined daily during the acclimatisation period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2-15. Mortality/viability was recorded once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.

Body weights were recorded on day -1 (prior to removal of food), day 1 (prior to administration) and on days 8 and 15.

All animals were killed at the end of the observation period by carbon dioxide asphyxiation, necropsied and examined macroscopically.

Results and discussion

Mortality: There were no mortalities.

Clinical observations: Slight ruffled fur was noted in all animals from the 30-minute reading to the 5-hour reading and persisted in one animal until test day 3. Hunched posture was also noted in the animals from the 1- or 2-hour reading to the 5-hour reading.

Body weight: The body weight of the animals was within the range commonly recorded for this strain and age.

Necropsy: No macroscopic findings were recorded at the scheduled necropsy.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (glyphosate technical material) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical material is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and the estimated oral LD₅₀ >5000 mg/kg bw is agreed.

Reference:

IIA, 5.2.1/15

Report:

(2011) Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure)

Data owner: Syngenta

Report No.: 10/218-001P

Date: 2011-04-15, not published

ASB2012-11392

Guidelines: OECD 425 (2008)
OPPTS 870.1100 (2002)
Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:
Identification: Glyphosate technical
Description: Technical, dry white powder
Lot/Batch #: 569753(BX20070911)
Purity: 96.3 %
Stability of test compound: Stable under storage conditions (room temperature range <30 °C), recertification date end August 2011
Vehicle and/or positive control: 0.5% Carboxymethylcellulose (CMC)
Test animals:
Species: Rat
Strain: RjHan:WI
Source: [REDACTED]
Age: 10-11 weeks
Sex: Female
Weight at dosing: 228-231 g
Acclimation period: At least 21 days
Diet/Food: ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany *ad libitum* (except for pre-dose fast)
Water: Tap water *ad libitum*
Housing: Individually in Type II. polypropylene/polycarbonate cages with Lignocel Bedding for Laboratory Animals
Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30 - 70 %
Air changes: 15 - 20/hour
12-hour light/dark cycle

Study design and methods:

In life dates: 2011-01-20 to 2011-02-10

Animal assignment and treatment:

In an acute oral toxicity study, a group of three, fasted, 10-11 week old, RjHan:WI female rats was given a single oral dose of glyphosate technical (96.3 % w/w glyphosate technical) at a concentration of 5000 mg/kg body weight by gavage. The test substance was diluted in vehicle (0.5 % carboxymethylcellulose) and administered at a dosing volume of 10 mL/kg.

Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. Treatment of an animal at the next dose was only performed when no significant clinical signs were noted in the previous animal.

All animals were examined for clinical signs once during the first 30 minutes, and at approximately 1, 2, 3, 4 and 6 hours after treatment on day 1 and once daily for 14 days thereafter.

Body weights were recorded on day -1 (prior to removal of food), day 0 (prior to administration) and on days 7 and 14.

All animals were exsanguinated under pentobarbital anaesthesia at the end of the observation period, necropsied and examined macroscopically.

Results and discussion

Mortality: There were no mortalities.

Clinical observations: No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw.

Body weight: The body weight of the animals was within the range commonly recorded for this strain and age.

Necropsy: No macroscopic findings were recorded at the scheduled necropsy.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (glyphosate technical) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate technical is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and evaluation is agreed.

Reference:

IIA, 5.2.1/16

Report:

(1995) Acute Toxicity Study of MON 0139 by Oral Administration in Mice.

Data owner: Monsanto

Monsanto Report No.: XX-95-205

Date: 1995-10-05, not published

ASB2012-11393

Guidelines:

JMAFF 59 NohSan No. 4200 (January 28, 1985)

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Identification:

MON 0139

Description:

Light yellow viscous solution

Lot/Batch #:

LBRV-11092

Purity:

62.34% (isopropyl amine salt of glyphosate)

Stability of test compound:	Stable under room temperature, expiry July, 1996
Vehicle and/or positive control:	Water for injection
Test animals:	
Species:	Mouse
Strain:	Crj:CD-1(ICR)
Source:	
Age:	6 weeks
Sex:	Male and female
Weight at dosing:	♂ 31.1 – 34.5 g; ♀ 22.2 – 26.2 g
Acclimation period:	Approximately 1 week
Diet/Food:	CRF-1 pelleted diet, sterilised by radiation (Oriental Yeast Co., Ltd.), <i>ad libitum</i> except during fasting prior to dosing
Water:	Tap water; <i>ad libitum</i>
Housing:	Plastic cages with wood chip bedding in groups of 5 (groups of 5 or 6 during quarantine/acclimation)
Environmental conditions:	Temperature: 23 ± 3 °C Humidity: 50 ± 20 % Air changes: 11 – 13 per hour Light cycle: 12 hour illumination per day

Study design and methods:

In life dates: 1995-08-16 – 1995-09-06

Animal assignment and treatment:

Preliminary study: During the quarantine/acclimatization period, a preliminary study was conducted using 9 males and 9 females. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage, adjusting the dose volume according to each dose level. Three male and female animals were dosed with 1000, 2000 or 5000 mg/kg body weight. The animals were fed again after administration, and had free access to water throughout the experimental period.

Main Study:

In the preliminary study, no deaths were observed in either sex in any of the dose groups. Based on these results, the dose level of 5000 mg/kg body weight was selected for the main test. The animals were ranked by individual body weights and randomly assigned to groups so as to ensure the homogeneity of group means as far as possible. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage. The animals in the 5000 mg/kg body weight group and control group were given 0.041 mL/10 g body weight of test article and 'water for injection', respectively. Each group consisted of 5 animals per sex. Animals were fed again after administration, and had free access to water throughout the experimental period.

The animals were observed frequently for the first 6 hours after administration, and then once daily for 14 days for mortality, signs of toxicity and abnormalities. Body weights were recorded prior to fasting, immediately before dosing, and on days 1, 2, 3, 7, 10 and 14 after dosing. A gross necropsy was performed on all animals at the time of terminal sacrifice at the end of the 14-day observation period.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No clinical signs of toxicity were observed.

Body weight: In males, a slight tendency toward retardation of body weight gain as compared with the control group was observed in the 5000 mg/kg body weight group from 7 days after administration (see Table B.6.2-5). In females, no compound-related changes were observed in the 5000 mg/kg body weight group.

Table B.6.2-5: Summary of male body weights

MON 0139 Dose (mg/kg body weight)	Days After Administration							
	0 ^a	0	1	2	3	7	10	14
0	34.4	32.6	34.5	35.2	35.4	37.3	38.1	38.8
5000	34.8	32.8	34.2	34.1	34.4	35.1	35.9	37.3

^a Prior to fasting

Necropsy: No abnormalities were observed.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (MON 0139) in mice was >5000 mg/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, MON 0139 is not to be classified for acute oral toxicity.

Comment by RMS:

The study is considered acceptable and the oral ED₅₀ > 5000 mg/kg bw in mice is agreed.

Reference: IIA, 5.2.1/17

Report: (1999) NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test

Study No. 7907

Date: 1999-09-16, unpublished

ASB2012-11394

Guidelines: US EPA Health Effects Test Guidelines, OPPTS 870.1100 (1998)

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:

Identification: NUP5a99 62 % glyphosate MOP

Description: clear viscous amber liquid

Lot/Batch #: Drum Sample E

Purity: 62 %

Stability of test compound: No data available

Vehicle and/or positive control: None

Test animals:

Species:	Rat
Strain:	Sprague-Dawley derived, albino
Source:	[REDACTED]
Age:	Not specified
Sex:	5 males and 5 females
Weight at dosing:	Young adult/males 227-254 grams and females 178-200 grams at experimental startg
Acclimation period:	14 days
Diet/Food:	Purina Rodent Chow #5012
Water:	Tap water, ad libitum
Housing:	singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 22-24 °C Humidity: not specified Air changes: not specified 12-hour light/dark cycle

Study design and methods

In life dates: 1999-08-03 – 1999-08-17

Animal assignment and treatment:

Prior to dosing, a group of animals was fasted for approximately 17.25 hours by removing feed from their cages. After weighing and clinical examination, ten (five male and five female) healthy rats were selected for test. Individual doses were calculated based on the initial bodyweights, taking into account the specific gravity (determined by PSL) of the test substance. Each animal received 5000 mg/kg of the test substance via gavage. Feed was replaced approximately 3.5 hours after dosing. The day of administration was considered Day zero of the study. Animals were weighed prior to test substance administration (initial) and again on Days 7 and 14 (termination). Clinical signs were recorded at 1,3 and 22 hours post-dosing and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and product safety labs central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma. All rats were euthanized via CO₂ inhalation on Day 14. Gross necropsies were performed on all animals.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Most females exhibited anogenital staining and two females exhibited soft feces or diarrhea, but recovered by Day 2.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The single dose acute oral LD₅₀ of NUP5a99 62 % glyphosate MUP is greater than 5000 mg/kg of bodyweight.

Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, NUP5a99 62 % glyphosate MOP is not to be classified for this endpoint.

Comment by RMS:

The study is considered acceptable and the estimated oral LD₅₀ is greater than 5000 mg/kg bw.

Comment by GTF on the first draft of the RAR (July 2013):

The Rat LD₅₀oral and Rat LD₅₀dermal are listed as >2000 mg/kg bw whereas the data presented concludes LD50-values above 5000 mg/kg. This would also be consistent with the values presented in summary Table B.6.13-2 in Volume 3 B.6.

RMS comment (August 2013):

To conclude on the LD50 value >2000 mg/kg bw seems to be more appropriate, because not all different batches were tested up to 5000 mg/kg bw. In any case Glyphosate is considered not to be classified as acute oral or dermal toxic according to GHS categories.

B.6.2.2 Acute percutaneous toxicity

Similarly, for the previous EU evaluation, a multitude of dermal toxicity studies in rats and rabbits were provided using glyphosate acid and its salts. Additional studies in rats with glyphosate acid were submitted for the current re-evaluation.

Table B.6.2-6: Summary of acute dermal toxicity studies with glyphosate acid

Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
Annex B- 5.2.2.1.1, Glyphosate Monograph [REDACTED], 1995 (Sanachem) TOX9650910	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	> 2000 (limit test)	splenomegaly, Liver: centri- lobular congestion
Annex B- 5.2.2.1.1, Glyphosate Monograph [REDACTED] 1994 (SIN) 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
Annex B- 5.2.2.1.1, Glyphosate Monograph [REDACTED] 1994 (Herbex) TOX9500246	Rat, Sprague Dawley	5/sex/2000	95	none	> 2000 (limit test)	No findings

Studies from the 2001 evaluation

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
Studies from the 2001 evaluation	Annex B- 5.2.2.1.1, Glyphosate Monograph [REDACTED] 1994 (ALKaloida) TOX9650143	Rat, Wistar	2/sex/0 5/sex/2000	97.2	water	> 2000 (limit test)	No findings
	Annex B- 5.2.2.1.1, Glyphosate Monograph [REDACTED] 1992 (Barclay) TOX9551813	Rat, Sprague- Dawley	5/sex/2000	> 97	none	> 2000 (limit test)	No findings
	Annex B- 5.2.2.1.1, Glyphosate Monograph [REDACTED] 1991 (FSG) TOX9551090	Rat, Wistar	5/sex/2500 5/sex/5000	96.8	Water (slurry)	> 5000	body weight loss
	Annex B- 5.2.2.1.1, Glyphosate Monograph [REDACTED], 1990 (AGC) TOX9551793	Rat, CD	5/sex/0 5/sex/3000 5/sex/5000 5/sex/8000	98.1	0.9 % saline	> 8000	No findings
Studies not reviewed in the 2001 evaluation	IIA 5.2.2/01 [REDACTED], 1989 (CHE) 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
	IIA 5.2.2/02 [REDACTED], 2009 (HAG) ASB2012- 11395	Rat, Sprague Dawley	5/sex/5050	96.4	water	> 5050	body weight loss in 1 male and 1 female
	IIA 5.2.2/03 [REDACTED] 1995 (ALS) ASB2012- 11396	Rat, SD	5/sex/2000	95.68	water	> 2000 (limit test)	No findings

Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
IIA 5.2.2/04 [REDACTED] 2009 (EXC) 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
IIA 5.2.2/05 [REDACTED] 2009 (HAG) ASB2012- 11398	Rat, CD	5/sex/2000	98.8	water	> 2000	No findings
IIA 5.2.2/06 [REDACTED] 2010 (HAG) ASB2012- 11399	Rat, CD	5/sex/2000	96.4	water	> 2000	No findings
IIA 5.2.2/07 [REDACTED] 2010 (HAG) ASB2012- 11400	Rat, CD	5/sex/2000	97.3	water	> 2000	No findings
IIA 5.2.2/08 [REDACTED], 2005 (HAG) ASB2012- 11401	Rat, Sprague Dawley	5/sex/5000	97.23	water	> 5000	No findings
IIA 5.2.2/09 [REDACTED] 2008 (HAG) ASB2012- 11402	Rat, Wistar Hannover r	5/sex/2000	98.05	water (for moisto- ning)	> 2000	No findings
IIA 5.2.2/10 [REDACTED], 2007 (NUF) ASB2012- 11403	Rat, HanRcc: WIST	5/sex/2000	95.1	PEG 300	> 2000 (limit test)	No findings
IIA 5.2.2/11 [REDACTED] 1996 (SYN) TOX2000- 1983	Rat	5/sex/2000	95.6	Moiste- ned with deionise d water	> 2000	Slight erythema in 1 ♂, small scabs in 1 ♀
IIA 5.2.2/12 [REDACTED] 2007 (SYN) ASB2012- 11404	Rat	5/sex/5000	96.1	Moiste- ned with purified water	> 5000	No findings

Studies not reviewed in the 2001 evaluation

WARNING: This document contains data of an EC evaluation data package and should not be read in isolation. Registration must not be done on the basis of this document.

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
	IIA 5.2.2/13 [REDACTED] 2011 (SYN) ASB2012- 11405	Rat	5/sex/5000	96.3	Moiste- ned with purified water	> 5000	No findings
Studies from the 2001 evaluation	Annex B- 5.2.2.1.2, Glyphosate Monograph [REDACTED] 1988 (MON) TOX9552325	Rabbit, NZW	5/sex/5000	97.8	Moiste- ned with saline	> 5000	Mortality (1 ♀); anorexia, diarrhea, soft stool

Table B.6.2-7: Summary of acute dermal toxicity studies with glyphosate salts

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Salt type Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
Studies from the 2001 evaluation	Annex B- 5.2.2.2.1, Glyphosate Monograph [REDACTED] 1995 (Sanachem) TOX9650910	Rat, Sprague Dawley	5/sex/2000	IPA 61.8	none	> 2000 (limit test)	severely congested lungs, splenomegaly, hepatomegaly with centrilobular congestion, subcapsuloar renal petechiae
	Annex B- 5.2.2.2.1, Glyphosate Monograph [REDACTED] 1989 (I.Pi.Ci.) TOX9551625	Rat, Wistar	5/sex/2000	IPA 62%	none	> 2000 (limit test)	Erythema maculate (1 ♂), scales (1 ♀)
	Annex B- 5.2.2.2.2, Glyphosate Monograph [REDACTED], 1987 (MON / CHE) TOX9552327	Rabbit NZW	5/sex/5000	Ammo nium salt 90.8	Moiste ned with saline	> 5000 (limit test)	Mortality (1 ♀); diarrhea, soft stool
Studies from the 2001 evaluation	Annex B- 5.2.2.2.2, Glyphosate Monograph [REDACTED] 1987 (MON / CHE) TOX9552328	Rabbit NZW	5/sex/5000	Sodiu m salt 80.3	Saline (1mL/g)	> 5000 (limit test)	Soft stool

Reference (Data owner)		Species Strain	Number of animals / Dose levels (mg/kg bw)	Salt type Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Main effects
	Annex B- 5.2.2.2.2, Glyphosate Monograph [REDACTED] 1981 (MON / CHE) TOX9552326	Rabbit NZW	5/sex/5000	IPA 65	none	> 5000 (limit test)	No findings

Tier II summaries are presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.

For details regarding studies reviewed during the 2001 EU evaluation please refer to the monograph.

Reference: IIA, 5.2.2/01

Report: [REDACTED] (1989) Acute dermal toxicity (limit) test in rats

Data owner: Cheminova A/S
Report No.: 5884
Date: 1989-06-21, not published
TOX9300328

Guidelines: OECD, EEC, EPA¹

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:

Identification: Glyphosate technical

Description: White powder

Lot/Batch #: 206-Jak-25-1

Purity: No data given in the report.

Stability of test compound: No data given in the report.

Vehicle and/or positive control: Water

Test animals: Rats

Species: Sprague-Dawley

Strain: [REDACTED]

Source: [REDACTED]

Age: 8-10 weeks

Guideline numbers are not specified in the report, however the study is compliant with OECD 402, EEC B3 and EPA 81-2 with the exception of a slightly different test item application procedure.

Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	♂ 212 - 240 g; ♀ 188 - 234 g
Acclimation period:	6 days
Diet/Food:	Expanded Rat and Mouse Maintenance Diet, <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	polypropylene cages with mesh floors in groups of 5 animals/cage.
Environmental conditions:	Temperature: 19 - 22 °C Humidity: 49 % 12-hour light/dark cycle

Study design and methods

In life dates: 1989-06-06 to 1989-06-21

Animal assignment and treatment:

A group of 5 male and 5 female rats was prepared by clipping the backs free of hair, approximately 24 hours before application of the test material. Care was taken to avoid abrading the skin. Glyphosate technical was administered dermally in a single application under occlusion at a dose level of 2000 mg/kg bw.

The test material was applied evenly onto gauze dressing, which was applied to the shaved back of each rat. The trunk of the rat was then encircled with a strip of non-irritating tape. After 24 hours the tape was removed and the skin was wiped with a water-dampened tissue to remove excess test material.

The rats were observed frequently on the day of dosing and once daily for 14 days following dosing. They were weighed immediately prior to dosing, 7 days after dosing and at sacrifice at the end of the 14-day observation period.

At the end of the observation period and sacrifice by carbon dioxide asphyxiation, each animal was subjected to a gross *post mortem* examination.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Clinical signs noted 30 minutes to 1 day after dosing included piloerection and reduced activity. Scab formation was noted at test sites 2 to 14 days after dosing.

Body weight: Body weight gains with the exception of one animal, which lost weight, were acceptable.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD₅₀ of the test material, glyphosate technical, in Sprague-Dawley Rats was estimated to be greater than 2000 mg/kg bw.

Comment by RMS:

In the study report no specific Test Guideline was mentioned, however, the study was considered acceptable. Under the condition of this study, the estimated dermal LD₅₀ > 2000 mg/kg bw is agreed.

Reference: IIA, 5.2.2/02
Report: (2009) Glyphosate – Acute Dermal Toxicity Study in Rats.
Data owner: Helm AG
Report No.: 12171-08
Date: 2009-03-11
GLP:
not published
ASB2012-11395
Guidelines: US EPA OPPTS 870.1200 (1998)
Deviations: Humidity was in the range of 30-86% instead of 30-70%. This deviation did not affect the study outcome.
GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate
Identification: Glyphosate Tech Grade Mixed 5-Batch
Description: White powder
Lot/Batch #: 080704-1 thru 5
Purity: 96.71 %
Stability of test compound: No data given in the report.
Vehicle and/or positive control: Deionised water
Test animals:
Species: Rat albino
Strain: Sprague-Dawley
Source:
Age: Approx. 8 weeks
Sex: 5 males and 5 females
Weight at dosing: Males: 299 - 348 g
Females: 189 - 207 g
Acclimation period: 5 days
Diet/Food: Formulab #5008 (PMI Feeds Inc.), *ad libitum*
Water: Tap water, *ad libitum*
Housing: Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30 - 70 %
Air changes: 10 - 12/hour
12-hour light/dark cycle

Study design and methods

In life dates: 2008-12-04 to 2008-12-18

Animal assignment and treatment:

One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 5050 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. Observations for evidence of dermal irritation were made at approximately 60 minutes after removal of wrappings, and on Days 4, 7, 11 and 14. On Day 14 after dosing, animals were euthanized by an overdose of CO₂. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: A single dermal administration of 5050 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

Body weight: Body weight gain was unaffected by the administration of the test substance, with the exception of 2 animals that lost or failed to gain weight during the study..

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD₅₀ of the test material (glyphosate) in rats was estimated to be greater than 5050 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate is not to be classified for acute dermal toxicity.

Comment by RMS:

The study is considered acceptable and the dermal LD₅₀ > 5000 mg/kg bw is agreed.

Reference:

IIA, 5.2.2/03

Report:

1995, HR-001: Acute dermal toxicity study in rats.

Data owner: Arysta LifeScience

Report No.: 94-0154

Date: 1995-03-14, not published

ASB2012-11396

Guidelines:

U.S. EPA FIFRA Guideline Subdivision F 81-2

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Glyphosate

Identification:

Glyphosate technical. Code HR-001

Description:

White crystal

Lot/Batch #:

Batch n° 940908-1

Purity:

95.68 %

Stability of test compound:

No data given in the report.

Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rat
Strain:	Specific pathogen free SD rats (Crj:CD)
Source:	
Age:	6 weeks
Sex:	Male and female
Weight at dosing:	248 – 268 g in males and 178 – 198 g in females
Acclimation period:	9 days
Diet/Food:	Certified diet pellet MF (Oriental yeast Co.)
Water:	Tap water, <i>ad libitum</i>
Housing:	Individual housing in suspended wire bottom, stainless steel cages.
Environmental conditions:	Temperature: 24.3 ± 0.3 °C Humidity: 53 - 55 % Air changes: 12 times/hour 12-hour light/dark cycle

Study design and methods

In life dates: 1995-01-31 – 1995-02-23

Animal assignment and treatment:

The test material was prepared as a suspension in deionised water. The suspension was applied on the shave skin of 5 males and 5 females specific pathogen free SD rats (Crj:CD) at a dose level of 2000 mg/kg. Mortality and clinical signs were recorded 1, 3 and 6 hours after administration and at least once daily thereafter until the termination of the 14-day observation period. All animals were weighed on the day of administration and on days 7 and 14 after administration. The surviving animals were euthanized after completion of the observation period (day 14) and examined for gross abnormalities. All animals were subjected to necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No clinical signs were noted in any animals of 0 and 2000 mg/kg groups.

Body weight: All animals gained their body weights on days 7 and 14 after administration.

Necropsy: There was no macroscopic abnormality in any surviving animals at final necropsy after completion of the observation period.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (glyphosate) in rats was estimated to be greater than 2000 mg/kg bw. However, the protocol of this study does not allow to confirm that glyphosate is not to be classified for acute oral toxicity based on the EU and the OECD Globally Harmonized System (GHS) classification criteria.

Comment by RMS:

The study is considered acceptable and the dermal LD₅₀ > 2000 mg/kg bw is agreed.

Reference: IIA, 5.2.2/04
Report: [REDACTED] 2009, Glyphosate Technical: Acute Dermal Toxicity Study in Rat.
[REDACTED] Data owner:
Excel
Report No.: C22875
Date: 2009-04-02, not published
ASB2012-11397
Guidelines: OECD 402 (1987)
Commission Regulation (EC) No 440/2008 (2008); method B.3
Deviations: Weight of female animals was outside of the range specified in the guideline (200-300 g).
GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material:
Identification: Glyphosate Technical
Description: Solid
Lot/Batch #: GI-1045
Purity: 96.66 %
Stability of test compound: No data given in the report. (Stable in purified water for 2 days.)
Expiry date: July 2010
Vehicle and/or positive control: Purified water
Test animals:
Species: Rat
Strain: HanRcc: WIST (SPF)
Source: [REDACTED]
Age: Males: 9 weeks
Females: 11 weeks
Sex: Males and female
Weight at dosing: 189.8 – 258.3 g
Acclimation period: 7 days
Diet/Food: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 61/08 (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland) ad libitum.
Water: Tap water, *ad libitum*
Housing: During acclimatisation in groups of five per sex in Makrolon type-4 cages with standard softwood bedding. Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, 4132 Muttensz / Switzerland) during treatment and observation.

Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30 - 70 %
Air changes: 10 - 15/hour
12-hour light/dark cycle

Study design and methods:

In life dates: 2009-01-20 to 2009-02-03

Animal assignment and treatment:

Single dose of 2000 mg/kg bw/day of test substance (glyphosate technical) was applied dermally to an area of clipped skin (approx. 10 % of body surface area) of five male and five female young adult rats. The treatment area was covered with a semi-occlusive dressing. Application volume was 4 mL/kg bw. Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dapped off with disposable paper towels. The animals were evaluated for effects on the day of dosing (Day 1) at 30 minutes and at 1, 2, 3 and 5 hours after application and once daily during days 2-15. Clinical observations, dermal findings, body weights and gross post mortem examinations were recorded.

Results and discussion

Mortality: No deaths occurred during the study.

Clinical observations: No systemic clinical signs were observed during the course of the study.

Body weight: A slight body weight loss (0.3-0.8 %) was observed in two females between acclimatisation and treatment start. The animals recovered until the end of the study. In spite of this body weight loss, the body weights of all animals were considered to be within the range commonly recorded for this strain and age.

Necropsy: No macroscopic findings were recorded at necropsy.

Skin observations: No local dermal signs were observed in any of the treated male animals. After removal of the patch, a very slight erythema was noted in four females on test day 4 and persisted up to test days 6, 11 or 12. Scaling was observed in the same four females on test day 4 and persisted up to test days 10, 11 and 12. Scabs were recorded in two females on test day 9 that persisted to test day P1.

Conclusion by the Notifiers

The dermal LD₅₀ of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical is not to be classified for acute dermal toxicity.

Comment by RMS:

According to the study report the body weight of female rats were in the range of 189.8 and 208.8 (mean 199.6) and therefore only slightly outside the suggested weight range. Under the present conditions the study is considered acceptable and the dermal LD₅₀ > 2000 mg/kg bw is agreed.

Reference:

IIA, 5.2.2/05

Report:

2009, Acute Dermal Toxicity Study of Glyphosate TC in CD Rats.

Guidelines: EC method B.3. (92/69/EEC), OECD 402 (1987) and US EPA OPPTS 870.1200 (1998)

Deviations: A personnel change in the Head of the Quality Assurance Unit did not affect the validity of the study outcome.

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate TC

Identification: Glyphosate technical grade

Description: White powder

Lot/Batch #: 20080801

Purity: 98.8 %

Stability of test compound: 2010-08-01

Vehicle and/or positive control: *Aqua ad iniectabilia*

Test animals:

Species: Rat albino

Strain: CD / CrI:CD(SD)

Source:

Age: Males: 51 days
Females: 65 days

Sex: 5 males and 5 females

Weight at dosing: Males: 224 - 234 g
Females: 200 - 217 g

Acclimation period: 5 days

Diet/Food: ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before administration

Water: Tap water, *ad libitum*

Housing: Individual housing in MAKROLON cages (type III plus) with granulated textured wood as bedding material.

Environmental conditions: Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 %
Air changes: 12 - 18/hour
12-hour light/dark cycle

Study design and methods

In life dates: 2009-02-04 to 2009-03-04

Animal assignment and treatment:

One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). The administration volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD₅₀ of the test material (glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute dermal toxicity.

Comment by RMS:

The study is considered acceptable and the dermal LD₅₀ > 2000 mg/kg bw is agreed.

Reference: IIA, 5.2.2/06

Report: [REDACTED] 2010, Acute Dermal Toxicity Study of Glyphosate TC in CD Rats.

Data owner: Helm AG

Report No.: 24876

Date: 2010-01-06, not published

ASB2012-11399

Guidelines: EC method B.3. (92/69/EEC), OECD 402 (1987) and US EPA OPPTS 870.1200 (1998)

Deviations: There were no deviations from the study plan.

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material: Glyphosate TC

Identification: Glyphosate technical grade

Description: White powder

Lot/Batch #: 2009051501

Purity: 96.4 %

Stability of test compound: 2011-05-15
Vehicle and/or positive control: *Aqua ad iniectabilia*
Test animals:
Species: Rat albino
Strain: CD / CrI:CD(SD)
Source: XX
Age: Males: approx. 7 weeks
Females: approx. 9 weeks
Sex: 5 males and 5 females
Weight at dosing: Males: 233 - 249 g
Females: 211 - 229 g
Acclimation period: 5 days
Diet/Food: ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before administration
Water: Tap water, *ad libitum*
Housing: Individual housing in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions: Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 %
12-hour light/dark cycle

Study design and methods

In life dates: 2009-10-15 to 2009-11-12

Animal assignment and treatment:

One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). The administration volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD₅₀ of the test material (glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute dermal toxicity.

Comment by RMS:

The study is considered acceptable and the dermal LD₅₀ >2000 mg/kg bw is agreed.

Reference: IIA, 5.2.2/07

Report: [REDACTED] 2010,
Acute Dermal Toxicity Study of Glyphosate TC in CD Rats.

Data owner: Helm AG

Report No.: 24604

Date: 2010-02-19, not published

ASB2012-11400

Guidelines: EC method B.3. (92/69/EEC), OECD 402 (1987) and US EPA OPPTS 870.1200 (1998)

Deviations: There were no deviations from the study plan.

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate TC

Identification: Glyphosate technical grade

Description: White powder

Lot/Batch #: 20090506

Purity: 97.3 %

Stability of test compound: May 2011

Vehicle and/or positive control: *Aqua ad iniectabilia*

Test animals:

Species: Rat albino

Strain: CD / CrI:CD(SD)

Source: [REDACTED]

Age: Males: approx. 7 weeks

Females: approx. 9 weeks

Sex: 5 males and 5 females

Weight at dosing: Males: 278 - 292 g

Females: 202 - 225 g

Acclimation period: 5 days

Diet/Food: ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before administration

Water: Tap water, *ad libitum*

Housing: Individual housing in MAKROLON cages (type III plus) with granulated textured wood as bedding material.

Environmental conditions: Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 %
12-hour light/dark cycle

Study design and methods

In life dates: 2009-10-26 to 2009-11-12

Animal assignment and treatment:

One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). The administration volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD₅₀ of the test material (glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute dermal toxicity.

Comment by RMS:

The study is considered acceptable and the dermal LD₅₀ > 2000 mg/kg bw is agreed.

Reference: IIA, 5.2.2/08

Report: [REDACTED] 2005 Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats – Limit Test.

[REDACTED]
Data owner: Helm AG

Report No.: 15275

Date: 2005-04-04, not published

ASB2012-11401

Guidelines: US EPA OPPTS 870.1200 (1998)

OECD 402 (1987).

Deviations: There were no deviations from the study plan.

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:	Glyphosate Acid Technical
Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	Test substance was expected to be stable for the duration of testing.
Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Rat albino
Strain:	
Source:	Ace Animals, Inc., Boyertown, PA, US
Age:	8 weeks
Sex:	5 males and 5 females
Weight at dosing:	Males: 231 - 264 g Females: 193 - 200 g
Acclimation period:	8 days
Diet/Food:	Purina Rodent Chow #5012, <i>ad libitum</i>
Water:	Filtered tap water, <i>ad libitum</i>
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 19-23 °C 12-hour light/dark cycle

Study design and methods

In life dates: 2004-05-05 to 2004-05-19

Animal assignment and treatment:

One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 5000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14.

On Day 14 after dosing, each animal was euthanized by an overdose of CO₂. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: A single dermal administration of 5000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD₅₀ of the test material (glyphosate acid technical) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate acid technical is not to be classified for acute dermal toxicity.

Comment by RMS:

The study is considered acceptable and the dermal LD50 > 5000 mg/kg bw is agreed.

Reference: IIA, 5.2.2/09

Report: [REDACTED] 2008, Acute Dermal Toxicity Study in Wistar Hannover Rats for Glyphosate Technical.

[REDACTED]
Data owner: Helm AG

Report No.: RF-3996.310.456.07

Date: 2008-07-04, not published

ASB2012-11402

Guidelines: OECD guideline 402 (1987)

Deviations: 1. The experimental phase initiation and experimental phase conclusion dates were updated.
2. One female rat weighed less than 200 g on the day of test item application.
These deviations did not affect the study outcome.

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material: Glyphosate Technical

Identification: Glyphosate Technical

Description: Solid

Lot/Batch #: 20070606

Purity: 98.05 %

Stability of test compound: No data given in the report.

Vehicle and/or positive control: Deionised water

Test animals:

Species: Rat albino

Strain: Wistar Hannover

Source: [REDACTED]

Age: 9 - 11 weeks

Sex: 5 males and 5 females

Weight at dosing: Males: 266 - 298 g
Females: 199 - 213 g

Acclimation period: 7 days
 Diet/Food: Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), *ad libitum*
 Water: Filtered drinking water, *ad libitum*
 Housing: Individual housing in polypropylene rodents cages with wire mesh tops and bedding material.
 Environmental conditions: Temperature: 22 ± 3 °C
 Humidity: 30 - 70 %
 Air changes: min. 10/hour
 12-hour light/dark cycle

Study design and methods

In life dates: 2007-09-11 to 2008-06-11

Animal assignment and treatment:

One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). Observations for mortality and clinical/behavioural signs of toxicity were made once within the first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were determined before the application of the test item (Day 0) and on days 7 and 14. On Day 14 after dosing, each animal was euthanized by an overdose of CO₂. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

Body weight: All animals gained the expected body weight, except for two females in the second week of the observation.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The dermal LD₅₀ of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical is not to be classified for acute dermal toxicity.

Comment by RMS:

The study is considered acceptable and the dermal LD₅₀ > 2000 mg/kg bw is agreed.

Reference: IIA, 5.2.2/10

Report: [REDACTED] 2007, GLYPHOSATE TECHNICAL (NUP05068):
 Acute dermal toxicity study in rats

[REDACTED]
 Data owner: Nufarm

[REDACTED] Study No.: B02283

Date: 2007-03-01, unpublished

ASB2012-11403

Guidelines: Japanese guideline Japanese Ministry of Agriculture, Forestry and

Fisheries (JMAFF),
 Guidelines for Preparation of Study Results, Acute Dermal Toxicity
 Studies, Guideline 2-1-2.
 Notification 12 NohSan No. 8147, as partly revised in 16-Shouan
 9260, on 16 March 2005.
 English translation by AGIS on 17 Oct 2005
 Directive 92/69/EEC, B.3. "Acute Toxicity-Dermal", July 31, 1992.
 OECD Guidelines for Testing of Chemicals, Section 4, Number 402
 "Acute Dermal Toxicity",
 adopted February 24, 1987.

Deviations: None
GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material:
 Identification: Glyphosate Technical (NUP 05068)
 Description: Solid
 Lot/Batch #: 200609062
 Purity: 95.1 %
 Stability of test compound: Stable under storage conditions.
 Vehicle and/or positive control: Polyethylene glycol 300 (PEG 300)
 Test animals:
 Species: Rat
 Strain: HanRcc:WIST (SPF)
 Source: RCC Ltd, Laboratory Animal Services
 Age: Males: 8 weeks
 Females: 11 weeks
 Sex: Male / Female
 Weight at dosing: 194.8-254.3g
 Acclimation period: 6 days
 Diet/Food: Pelleted standard Provimi Kliba 3433 rat/mouse
 maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-
 4303 Kaiseraugst/ Switzerland) ad libitum
 Water: Tap water, ad libitum
 Housing: During acclimatization in groups of five per sex in
 Makrolon type-4 cages with standard softwood bedding.
 Individually in Makrolon type-3 cages with standard
 softwood bedding ("Lignocel", Schill AG, CH-4132
 uttENZ) during treatment and observation.
 Environmental conditions: Temperature: 22 ± 3 °C
 Humidity: 30-70 %
 Air changes: 10 - 15/hour
 12-hour light/dark cycle

Study design and methods

In life dates: 12-DEC-2006 to 02-JAN-2007

Animal assignment and treatment:

One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface. Only those animals without injury or irritation on the skin were used in the test. On test day 1, the test item was applied at a dose of 2000 mg/kg body weight evenly on the intact skin with a syringe and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an elastic adhesive bandage. Application volume/kg body weight: 6 mL. Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the reaction sites were assessed. The fur of all animals was shaved on test days 4 and 9 just after the assessment of the reaction to facilitate the skin reading for the next day. Rationale: Dermal administration was used as this is one possible route of human exposure during manufacture, handling and use of the test item.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No systemic or local signs of toxicity were observed during the study period.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The median lethal dose of glyphosate technical (NUP 05068) after single dermal administration to rats of both sexes, observed over a period of 14 days is: Dermal LD₅₀ (rat) > 2000 mg/kg body weight.

Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for this end point.

Comment by RMS:

The study is considered acceptable and the dermal LD₅₀ > 2000 mg/kg bw is agreed.

Reference:

IIA, 5.2.2/11

Report:

1996, Glyphosate Acid: Acute Dermal Toxicity Study In The Rat.

Data owner: Syngenta

Report No.: /P/4664

Date: 1996-08-23, not published

TOX2000-1983

Guidelines:

OECD guideline 402 (1987), OPPTS 870.1200 (1998): 92/69/EEC B.3 (1992)

Deviations:

None

GLP:

yes

Materials and methods

Test animals:

Housing: Individually in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study.

Acclimatisation period:	At least 6 days
Diet:	Diet (PCD), supplied by Special Diet Services Limited, Witham, Essex, UK <i>ad libitum</i> .
Water:	Mains water <i>ad libitum</i>
Environmental conditions:	Temperature: 21±2 °C Humidity: 40-70 % Air changes: Approximately 25-30/hour Photoperiod: 12 hours dark / 12 hours light

Study design and methods

In-life dates: Start: 16 March 1995 End: 30 March 1995

Animal assignment and treatment: In an acute dermal toxicity study, a group of five male and five female, young adult Alpk:AP₁SD (Wistar-derived) rats were given a single dermal application of 2000 mg/kg bw of glyphosate acid.

Sixteen to thirty-two hours before application, the hair was removed by clipping from an area on the dorso-lumbar region of each rat (approximately 10 cm x 5 cm). The appropriate amount of test substance was moistened to a dry paste with 0.6-0.8 mL of deionised water. Approximately half the application site was covered by test substance (equivalent to 20.0-21.9 mg/cm² for males and 16.2-17.3 mg/cm² for females). The application site was covered with a 4-ply gauze patch (approximately 7 cm x 7 cm) and kept in place for 24 hours using an occlusive dressing. The gauze patch was covered by a piece of plastic film (7 cm x 7 cm), held in place using adhesive bandage (25 cm x 7.5 cm) secured by two pieces of PVC tape. At the end of the 24 hour contact period, the dressings were carefully removed and the skin cleansed of any residual test substance using clean warm water.

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic

toxicity once between one and four hours of dosing. Subsequent observations were made daily, up to day 15.

The animals were weighed immediately before dosing (day 1) and on days 3, 8, 8 and 15. All animals were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera.

Statistics: The dermal LD₅₀ was estimated (limit test, no mortalities).

Results and discussion

Mortality: There were no mortalities.

Clinical observations: There were no significant signs of systemic toxicity (only urinary incontinence due to bandaging). There were practically no signs of skin irritation. One male showed slight erythema on days 2 and 3 and one female had small scabs from day 3 to 8.

Body weight: Two males and three females lost weight initially, but all had exceeded their initial weight by day 5, and except for one female, continued to gain weight throughout the remainder of the study. One female lost weight slightly from day 5.

Necropsy: Red mottled lungs were seen in one female. This is a common spontaneous finding in rats of this age and strain and is considered not to be treatment-related.

Conclusion by the Notifiers:

The acute dermal LD₅₀ of glyphosate acid is in excess of 2000 mg/kg to male and female rats (limit test, no mortality).

Comment by RMS:

The study is considered acceptable and the dermal LD₅₀ > 2000 mg/kg bw is agreed.

Reference:

IIA, 5.2.2/12

Report:

2007. Glyphosate technical material: Acute dermal toxicity study in rats.

Data owner: Syngenta

Report No.: B02766

Date: 2007-02-08, not published

ASB2012-11404

Guidelines:

OECD 402 (1987), JMAFF 12 NohSan 8147 (2000), US EPA Health Effects Test Guidelines, OPPTS 870.1200 (1998)

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Glyphosate technical material

Description:

Technical, white powder

Lot/Batch number:

0507

Purity:

96.1 % w/w glyphosate acid

CAS#:

Not reported

Stability of test compound:

Re-certification date August 2008. Stable under storage conditions (room temperature range 20±5°C, protected from

light and humidity).

Vehicle and/or positive control: The test item was applied moistened with purified water before application.

Test animals:

Species: Rat

Strain: HanRcc:WIST (SPF)

Age/weight at dosing: 8 weeks (males), 11 weeks (females) / 247.0-222.7 g (males),- 191.3-204.2 g (females)

Source: [REDACTED]

Housing: Individually in Makrolon type-3 cages with standard softwood bedding

Acclimatisation period: 7 days

Diet: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) *ad libitum*

Water: Community tap water *ad libitum*.

Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30-70 %
Air changes: 10-15 air changes per hour
Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 07 December 2006 End: 22 December 2006

Animal assignment and treatment: In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female HanRcc:WIST (SPF) rats were treated with glyphosate technical material (96.1 % w/w glyphosate technical) at 5000 mg/kg by dermal application. The test item was moistened with purified water before application. The application period was 24 hours.

One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface. Only those animals without injury or irritation on the skin were used in the test.

A single animal of each sex was treated first. As no deaths and neither severe local effects nor severe systemic symptoms were observed after the 24-hour exposure, the test was completed using the four remaining male and female animals for an exposure period of 24 hours.

On test day 1, the mass of the dose (5000 mg/kg) was calculated for each animal on the basis of its body weight. The appropriate amount of test item was weighed out on a suitable precision balance into a plastic weighing boat and moistened to a dry paste with a minimum amount of purified water (0.5 to 0.6 mL) to allow good skin contact. The dry paste was applied evenly on the intact skin of the clipped area and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with tape. The area of skin covered by the test item was approximately 8 cm² for the males and females.

Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the reaction sites were assessed.

The fur of all males and females was shaved, on test day 6 (female no. 2), on test days 5 and 9 (male no. 1), on test days 4 and 8 (males nos. 3 - 6 and females nos. 7 - 10) just after the assessment of the reaction to facilitate the skin reading for the next day.

The animals were checked daily for mortality/viability during the acclimatization period, at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.

Clinical observations were recorded daily during the acclimatisation period, at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on test day 1 and once daily during days 2-15. The animals were examined daily for local signs at the application site.

Body weights were recorded on test days 1 (prior to administration), 8 and 15.

All animals were killed at the end of the observation period by Carbon dioxide asphyxiation and discarded after macroscopic examinations were performed. No organs or tissues were retained.

Statistics: Not applicable (limit test, no mortalities).

Results and discussion

Mortality: There were no mortalities.

Clinical observations: No systemic signs or local signs of irritation were noted during the course of the study.

Body weight: The body weights of the animals were within the range commonly recorded for this strain and age.

Necropsy: No macroscopic findings were recorded at the scheduled necropsy.

Conclusion by the Notifiers:

The acute dermal LD₅₀ of glyphosate technical material after a single dermal administration to male and female HanRcc:WIST (SPF) rats, observed over a period of 14 days was greater than 5000 mg/kg body weight (limit test, no mortalities).

Comment by RMS:

The study is considered acceptable and the dermal LD50 is estimated greater than 5000 mg/kg bw

Reference:

IIA, 5.2.2/13

Report:

2011, Glyphosate technical: Acute dermal toxicity study in rats; Final report amendment 1.

Data owner: Syngenta

Report No.: 10/218-002P

Date: 2011-04-13, not published

ASB2012-11405

Guidelines:

OECD 402 (1987); OPPTS 870.1200 (1998); EC 440/2008 (2008)

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test Material:

Glyphosate technical

Description:

Technical, dry white powder

Lot/Batch number:

569753 (BX20070911)

Purity:

96.3 % w/w Glyphosate technical

CAS#:

Not reported

Stability of test compound: Stable under storage conditions (room temperature range <30 °C), recertification date end August 2011

Vehicle and/or positive control: None (tested as supplied)

Test animals:

Species: Rat

Strain: RjHan:(WI) Wistar

Age/weight at dosing: Young adult / 220-259 g

Source: [REDACTED]

Housing: Individually in Type II. polypropylene/polycarbonate cages with Lignocel Bedding for Laboratory Animals

Acclimatisation period: 6 days

Diet: ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany *ad libitum*

Water: Tap water *ad libitum*

Environmental conditions: Temperature: 20.7-24.0 °C
Humidity: 39-65 %
Air changes: 15-20 air changes per hour
Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 06 October 2010 End: 20 October 2010

Animal assignment and treatment: In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female RjHan:(WI) Wistar rats were treated with a single administration of glyphosate technical (96.3 % w/w glyphosate technical) at 5000 mg/kg by dermal application. The test item was applied as supplied. The application period was 24 hours, followed by a 14-day observation period.

The backs of the animals were shaved (approximately 10 % area of the total body surface) approximately 24 hours prior to the treatment. Only those animals without injury or irritation on the skin were used in the test. On test day 0, the test item was applied at a single dose of 5000 mg/kg body weight applied uniformly over the skin and remained on the skin throughout a 24- hour exposure period. The test item was moistened with water to ensure good contact with the skin. Sterile gauze pads were placed on the skin of rats at the site of application. These gauze pads were kept in contact with the skin by a patch with adhesive hypoallergenic plaster. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24 hours. At the end of the exposure period, residual test item was removed, using body temperature water.

A clinical examination was performed on the day of treatment, at 1 and 5 hours after the application of the test item, and once each day for 14 days thereafter.

Observations included the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behaviour pattern. Particular attention was directed to the observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

The body weight of all animals was recorded on Day 0 (beginning of the experiment) and on Days 7 and 14.

All animals were anaesthetised with Euthasol®40 % and exsanguinated. After examination of the external appearance, the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. Any gross macroscopic findings were recorded.

Statistics: Not applicable (limit test, no mortalities).

Results and discussion

Mortality: There were no mortalities.

Clinical observations: There were no clinical signs noted in any animals throughout the study.

No treatment related skin irritation was observed in any animal throughout the study.

Body weight: There were no effects on body weight and body weight gain during the observation period.

Necropsy: No macroscopic findings were recorded at the scheduled necropsy.

Conclusion by the Notifiers

The acute dermal LD₅₀ of glyphosate technical after a single dermal administration to male and female RjHan:WI Wistar rats, observed over a period of 14 days was greater than 5000 mg/kg body weight (limit test, no mortalities).

Comment by RMS:

The study is considered acceptable and the dermal LD₅₀ > 5000 mg/kg bw is agreed.

Comment by GTF on the first draft of the RAR (July 2013):

The Rat LD₅₀oral and Rat LD₅₀dermal are listed as >2000 mg/kg bw whereas the data presented concludes LD₅₀-values above 5000 mg/kg. This would also be consistent with the values presented in summary Table B.6.13.2 in Volume 3 B6.

RMS comment (August 2013):

To conclude on the LD₅₀ value >2000 mg/kg bw seems to be more appropriate, because not all different batches were tested up to 5000 mg/kg bw. In any case glyphosate is considered not to be classified as acute oral or dermal toxic according to GHS categories.

B.6.2.3 Acute inhalation toxicity

For the previous EU evaluation, a multitude of acute inhalation toxicity studies in rats were provided using glyphosate acid and its IPA salt. For the current re-evaluation, additionally eleven inhalation studies with glyphosate acid and two with glyphosate salts (one for potassium salt) were submitted.

Table B.6.2-8: Summary of acute inhalation toxicity studies with glyphosate acid

Reference (Data owner)	Species Strain	Number of animals / Dose level (mg/L)	Purity (%)	Vehicle	LC ₅₀ (mg/L air)	Main effects

Reference (Data owner)		Species Strain	Number of animals / Dose level (mg/L)	Purit y (%)	Vehicle	LC ₅₀ (mg/L air)	Main effects
Studies from the 2001 evaluation	Annex B- 5.2.3.1, Glyphosate Monograph ██████████ 1995 (Herbex) TOX9500247	Rat, Sprague Dawley	5/sex/5.35	95	Compres sed air; 4 h nose- only	> 5.35	Wet fur, hunched posture, piloerection, incidents of decreased respiratory rate, ptosis, brown stained fur (head)
	Annex B- 5.2.3.1, Glyphosate Monograph ██████████, 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
	Annex B- 5.2.3.1, Glyphosate Monograph McDonald & A ██████████, 1989 (CHE) TOX9552329	Rat, Sprague Dawley	5/sex/4.98	98.6	Dust aerosol; 4 h snout only	> 4.98	No adverse findings
Studies not reviewed in the 2001 evaluation	IIA, 5.2.3/01 ██████████, 2010 (HAG) 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
	IIA 5.2.3/02 K ██████████ 1995 (ALS) 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)
	IIA 5.2.3/03 ██████████ 2009 (EXC) 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
	IIA 5.2.3/04 ██████████, ASB2012- 11409	Rat, CD	5/sex/5.12 (dust)	98.8	4h (MMAD : 6.62µm)	> 5.12 (limit test)	Slight dyspnoea and ataxia during exposure
	IIA 5.2.3/05 ██████████, 2010 (HAG) ASB2012- 11410	Rat, CD	5/sex/5.02	96.4	4h (MMAD : 4.2µm)	> 5.02	Slight dyspnoea, slight ataxia and slight tremor during exposure until 3 h after exposure

Reference (Data owner)		Species Strain	Number of animals / Dose level (mg/L)	Purit y (%)	Vehicle	LC ₅₀ (mg/L air)	Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.2.3/06 [REDACTED] 2009 (HAG) ASB2012- 11411	Rat, Sprague- Dawley	5/sex/2.24	96.4	4 h (MMAD : 2.6 µm)	>2.24 (limit test)	No findings
	IIA 5.2.3/07 [REDACTED] 2005 (HAG) ASB2012- 11412	Rat, Sprague- Dawley	5/sex/2.04	97.23	4 h (MMAD : 2.5 µm)	> 2.04 (limit test)	No findings
	IIA 5.2.3/08 [REDACTED] 2008 (HAG) ASB2012- 11413	Rat, Wistar Hannove r	5/sex/5.21	98.05	4 h (MMAD : 18.2- 19.9µm)	> 5.21 (study not accepta- ble)	Wheeze and dyspnoea
	IIA 5.2.3/09 [REDACTED] 2007 (NUF) ASB2012- 11414	Rat, albino	5/sex/3.252 (highest techn. attain.)	95.1	4 h (MMAD : 2.95 – 3.05 µm)	> 3.252	Salivation in males, breathing effects in both sexes, body weight loss
	IIA 5.2.3/10 [REDACTED] 1996 (SYN) 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
	IIA 5.2.11/ [REDACTED], 2011 (SYN) 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.

Table B.6.2-9: Summary of acute inhalation toxicity studies with glyphosate salts

Reference (Data owner)	Species Strain	Number of animals / Dose level (mg/L)	Purit y (%)	Vehicle	LC ₅₀ (mg/L air)	Main effects
Studies from the 2001 evaluation Annex B- 5.2.3.2, Glyphosate Monograph [REDACTED] 1994 (MON/CHE) TOX9552331	Rat, Sprague Dawley	5/sex/4.24 (max. attainable conc.)	IPA 62	aerosol; 4 h nose- only	> 4.24	Lung: dark areas or multiple dark foci in 6 of 10 rats

Reference (Data owner)		Species Strain	Number of animals / Dose level (mg/L)	Purit y (%)	Vehicle	LC ₅₀ (mg/L air)	Main effects
	Annex B- 5.2.3.2, Glyphosate Monograph [REDACTED], 1989 (I. Pl. Ci.) TOX9551626	Rat, Wistar	5/sex/4.1 5/sex/4.42 5/sex/6.49	IPA 62% in water	Nebu- lised; 4 h nose-only	> 6.49	Nose bleeding, ruffled fur
	Annex B- 5.2.3.2, Glyphosate Monograph [REDACTED] 1988 (MON / CHE) TOX9552332	Rat, Sprague Dawley	5/sex/1.9 (max. attainable conc.)	Amm onium salt 85.5	Aerosol / vapor; 4 h whole body	> 1.9	Hyperactivity, perinasal encrustation
	Annex B- 5.2.3.2, Glyphosate Monograph [REDACTED] 1987 (MON / CHE) TOX9552330	Rat, Sprague- Dawley	5/sex/1.3 (max. attainable conc.)	IPA 53.8	Aerosol / vapor, 4 h whole body	> 1.3	Mortality (1♀); yellow/brown nasal discharge, local and/or generalised hairloss, slight decreased body weight
Studies not reviewed in the 2001 evaluation	IIA 5.2.3/12 [REDACTED], 1999 (NUF) ASB2012- 11416	Rat, Sprague Dawley	Isopropylamine glyphosate 5/sex/2.08	IPA 62	air / 4 h nose-only (MMAD 2.6 µm)	> 2.08	During exposure: ocular and nasal discharge, hunched posture and hypoactivity. After exposure no findings
	IIA 5.2.3/13 [REDACTED] 2004 (MON) ASB2012- 11417	Rat. Sprague- Dawley	K-salt of glyphosate 5/sex/2.21 5/sex/5.27	K-salt 57.8% (= 47.2% glyph o- sate)	Aerosol / 4 h nose- only (MMADs: 2.9 µm, 3.8 µm)	> 5.27	2.21 mg/L: congested brea- thing, dark material around eyes and nose, few faeces 5.27 mg/L: congested brea- thing,

Tier II summaries are presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.

For details regarding studies reviewed during the 2001 EU evaluation please refer to the monograph

Reference:

IIA, 5.2.3/01

Report:

[REDACTED] 2010, Acute Inhalation Toxicity Study of Glyphosate
TC In Rats

Data owner: Helm AG

Report No.: 24603
Date: 2010-06-03, not published
ASB2012-11406
Guidelines: EC method B.2, OECD 403, EPA Health Effects Test Guidelines, OPPTS 870.1300
Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:
Identification: Glyphosate TC
Description: White powder
Lot/Batch #: 20090506
Purity: 97.3 %
Stability of test compound: At room temperature in the dark stable until May 2011.
Vehicle and/or positive control: none
Test animals:
Species: Rat
Strain: CD/Crl:CD (SD)
Source: [REDACTED]
Age: approx. 7-9 weeks
Sex: Males and females
Weight at dosing: ♂ 234 - 270 g; ♀ 208 - 244 g
Acclimation period: At least 5 days
Diet/Food: ssniff RIM-H V1 534 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* (except 16 h before exposure)
Water: tap water, *ad libitum*
Housing: In groups of 2-3 animals per cage in Makrolon type III plus cages with granulated textured wood bedding.
Environmental conditions: Temperature: 22 ± 3 °C
 Humidity: 55 ± 15 %
 Air changes: no data
 12 hours light/dark cycle

Study design and methods

In life dates: 2010-02-02 to 2010-02-16

Test atmosphere generation:

A dust atmosphere was produced from the test material using a rotating brush dust generator and compressed air.

Exposure chamber conditions:

The cylindrical exposure chamber had a volume of approximately 40 L. The actual dust concentration was measured four times gravimetrically with an air sample filter (Minisart SM 17598; 0.45 µm) and pump (Vacuubrand, MZ 2C, Vacuubrand, Germany) controlled by a rotameter. Dust samples were taken once every hour during the exposure. For that purpose, a probe was placed close to the animals' noses in the inhalation chamber and air was sucked through the air sample filter at a constant flow of air of 5 L/min for 1 minute. The filters were weighed before and after sampling on an analytical balance (accuracy 0.1 mg). Chamber airflow rates ranged from 800 to 900 L/h, providing ≥ 12 air changes per hour.

Particle size distribution:

A Malvern Spraytec Lasersystem (Malvern Instruments, Germany) was employed for the determination of the particle size distribution of the particle diameter (volume) in the exposure air. The particle size distribution of the test atmospheres was measured using a cascade impactor two times during the exposure period. The results were as follows:

Table B.6.2-10: Details of test atmosphere

Mean achieved actual concentration (HPLC)	Actual concentration (gravimetric method)	MMAD	GSD	Respirable amount particle size $\leq 4 \mu\text{m}$	
(mg/L air)	(mg/L air)	(µm)		(mg/L air)	(%)
5.18	5.05	4.633	3.02	1.08	20.8

MMAD = mean mass median aerodynamic diameter

GSD = geometric standard deviation

The generated dust had a mass median aerodynamic diameter (MMAD) of 4.633 µm as determined with a cascade impactor. The Geometric Standard Deviation (GSD) of the MMAD was calculated as 3.02. No smaller MMAD and GSD could be obtained with the test item supplied.

Animal assignment and treatment:

A group of five fasted rats per sex received the test material at a dose level of 5.18 mg/L using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure. Observations for mortality and clinical/behavioural signs of toxicity were made at least once per day for 14 days. Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanised and all study animals were subjected to gross necropsy.

Results and discussion

Mortality: No deaths occurred.

Clinical observations: Clinical signs of toxicity included slight tremor and slight dyspnoea immediately until 3 hours after end of exposure.

Body weight: All animals gained the expected body weight.

Necropsy: No pathological findings were noted at necropsy.

Conclusion by the Notifiers

The acute inhalation LC_{50} for the test substance glyphosate TC was calculated to be greater than 5.18 mg/L. According to EU and OECD Globally Harmonised System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity.

Comment by RMS:

The MMAD and GSD are greater than recommended in OECD 403, however the explanation given by the authors is acceptable. Under the present conditions the study is considered acceptable and the acute inhalation $LC_{50} > 5$ mg/L air/4 hours is agreed.

Reference: IIA, 5.2.3/02
Report: [REDACTED] 1995,
HR-001: Acute inhalation toxicity study in rats.
[REDACTED]
Data owner: Arysta LifeScience
Report No.: 94-0155, not published
ASB2012-11407
Guidelines: U.S. EPA FIFRA Guideline Subdivision F
Deviations: None
GLP: yes
Acceptability: Please see comment RMS

Materials and methods

Test material:
Identification: Glyphosate TC
Description: Whitish crystals
Lot/Batch #: T-941209
Purity: 97.56 %
Stability of test compound: Not mentioned in the report.
Vehicle and/or positive control: None
Test animals:
Species: Rat
Strain: F344/DuCrj
Source: [REDACTED]
Age: 8 weeks
Sex: Males and females
Weight at dosing: ♂ 176 - 187 g; ♀ 138 - 144 g
Acclimation period: 8 days
Diet/Food: ssniff RIM-H V1 534 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* (except 16 h before exposure)
Water: tap water, *ad libitum*
Housing: By group of 5 animals of the same sex in stainless steel wire cages during pre- and post-exposure periods.
Individually in stainless steel wire cages during exposure.
Environmental conditions: Temperature: 22 ± 2 °C
Humidity: 55 ± 15 %
Air changes: 10/hour
12 hours light/dark cycle

Study design and methods

In life dates: 1995-03-28 to 1995-04-20

Test atmosphere generation:

The dust was generated by a turn-table type dust feeder with compressed air from air compressor. The compressed air was supplied to the dust feeder through an air filter. The air was introduced into the chamber as diluting air after filtering it through a HEPA filter.

Exposure chamber conditions:

The nominal atmospheric concentration of HR-001 was calculating by dividing the total amount of the test substance supplied to the duct feeder during the 4-hour exposure by the total air volume delivered during the exposure.

The actual atmospheric concentration was measured gravimetrically.

Particle size distribution:

The results for the air samples taken for the determination of particle size distribution are given in Table B.6.2-11.

Table B.6.2-11: Particulate size distribution

Exposure group	Time of sampling	Analytical concentration	Particle size	
mg/L	(min)	(mg/L)	MMAD ² (μm)	σg ³
5.48	60	6.54	5.9	1.6
	120	4.80		
	180	5.11	4.6	1.8
	Mean	5.48	4.8	1.7
	S.D. ⁴	0.93	0.3	0.1

MMAD : Mass median aerodynamic diameter

σg: geometric standard deviation

SD: Standard deviation

The results showed that particles size of HR-001 expressed as the mean value of NMAD during the exposure was 4.8 μm (σg = 1.7). Thus more than 91 % of the test substance dust consisted of particles present consisted of inhalable particles

Animal assignment and treatment:

Groups of 5 male and 5 female specific pathogen free Fisher rats (F344/DuCrj) were exposed (whole-body) continuously for 4 hours to test substance mist containing concentrations of HR-001 at 5.48 mg/l. The flow rate was stable at approximately 100 l/min. Mortality and signs of reaction to treatment were recorded during a subsequent 14-day observation period. All animals were observed for clinical signs at 2 hours after the initiation of exposure, immediately and at 2 hours after the termination of exposure. In addition, animals were observed for lethality at 4 hours after the termination of exposure. All animals were weighed shortly before the exposure and on days 7 and 14. The surviving animals were euthanized on the following day (day 15). All animals were subjected to necropsy.

Results and discussion

Mortality: There were no deaths in either sex at the tested concentration 5.48 mg/L.

² Mass median aerodynamic diameter

³ geometric standard deviation

⁴ Standard deviation

Clinical observations: No notable serious changes were observed as clinical signs. Wetted and soiled fur in the periorcular and nasorostral regions were not considered to be particularly caused by HR-001 because the changes were slight in degree and are frequently observed in the acute inhalation toxicity study.

Body weight: All animals gained weights, reflecting their good healthy conditions.

Necropsy: No abnormalities were observed in any animal of either sex at necropsy.

Conclusion by the Notifiers

The acute inhalation LC_{50} for the test substance glyphosate HR-001 was calculated to be greater than 5.48 mg/L. According to EU and OECD Globally Harmonised System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity.

Comment by RMS:

The study is considered acceptable and the acute inhalation LC_{50} of 5 mg/L air/4 hours is agreed.

Reference: IIA, 5.2.3/03

Report: [REDACTED] 2009, Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat

[REDACTED]
Data owner: Excel

Report No.: 2743/0001

Date: 2009-06-22, not published

ASB2012-11408

Guidelines: OECD 403 (1981)

Commission Regulation (EC) No 440/2008 (2008), method B.2 (2008)

Deviations: Particle size diameter larger than required by the test guidelines.

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:

Identification: Glyphosate Tech

Description: White powder

Lot/Batch #: GI-1045

Purity: 96.66 %

Stability of test compound: Expiration in July 2010.

Vehicle and/or positive control: Not relevant

Test animals:

Species: Rat

Strain: HsdRccHanTM : WIST

Source: [REDACTED]

Age: Approx. 8 - 12 weeks

Sex: Male and female

Weight at dosing:	178 - 350 g
Acclimation period:	5 days
Diet/Food:	With the exception of the exposure period, free access to food (Harlan 2014 Rodent Diet, Harlan UK Limited, Oxon, UK) was allowed throughout the study.
Water:	With the exception of the exposure period, free access to drinking water was allowed throughout the study.
Housing:	Housed in groups of five by sex in solid-floor polupropylene cages with stainless steel lids, furnished with softwood flakes (Datesand Ltd., Cheshire, UK) and provided with environmental enrichment items: wooden chew blocks and cardboard “fun tunnels” (Datesand Ltd., Cheshire, UK).
Environmental conditions:	Temperature: 19 – 25 °C Humidity: 30 – 70 % Air changes: At least 15/hour 12-hour light/dark cycle

Study design and methods

In life dates: 2009-05-06 to 2009-05-12

Animal assignment and treatment:

Five male and female rats were exposed to one dose level of an aerosol atmosphere of glyphosate. The single 5 mg/L four hour exposure was “nose only” at a mean actual concentration of 5.04 ± 0.37 mg/L (nominal concentration was 27.3 mg/L).

Operational conditions (flow rate, oxygen levels, temperature, and humidity in the inhalation systems) were checked throughout the exposure period. All animals were observed for clinical signs at hourly intervals during exposure, immediately on removal from the restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for 14 days. Individual body weights were recorded prior to treatment on the day of exposure and on Days 7 and 14. At the end of the fourteen day observation period the animals were killed by intravenous overdose of sodium pentobarbitone. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.

The chamber flow rate was maintained at 45 L/min providing 90 air changes per hour.

Results and discussion

Test atmosphere

The particle size analysis of the atmosphere drawn from the animals’ breathing zone, was as follows:

Table B.6.2-12: Details of test atmosphere

Mean Achieved Atmosphere Concentration (mg/L)	Mean Mass Median Aerodynamic Diameter (μ m)	Inhalable Fraction (% <4 μ m)	Geometric Standard Deviation
5.04	5.25	41.1	3.35

It is noted that the achieved particle size is larger than required by the test guidelines.

During characterisation, changes were made to the generation system (addition of particle sizes separator) and grinding techniques in an attempt to increase the inhalable portion of the test material. However, this reduced the achieved concentration, and therefore, also reduced the actual concentration of particles $<4\ \mu\text{m}$. It was, therefore, preferable to expose the animals to a higher concentration of test material, even though this also increased the mean mass median aerodynamic diameter, as this resulted in the animals being exposed to the highest possible concentration of particles $<4\ \mu\text{m}$.

Mortality: There were no mortalities during the study.

Clinical observations: Signs of hunched posture and piloerection are commonly seen in animals for short periods on removal from the chamber following 4-hour inhalation studies. Wet fur is commonly recorded both during and for a short period after exposure. These observations are considered to be associated with the restraint procedure and, in isolation, are not indicative of toxicity.

In addition to the observations considered to be due to the restraint procedure, increased respiratory rate was noted in all animals during exposure, on removal from the chamber and one hour post-exposure.

Body weight: Normal bodyweight development was noted during the course of the study.

Necropsy: No macroscopic abnormalities were detected at necropsy.

Conclusion by the Notifiers

The inhalation LC_{50} (4 hours) of the test material (glyphosate tech) in rats was estimated to be greater than 5.04 mg/L. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate tech is not to be classified for acute inhalation toxicity.

Comment by RMS:

Under the present conditions the study is considered acceptable and the acute inhalation $\text{LC}_{50} > 5\ \text{mg/L air/4 hours}$ is agreed.

Reference:

IIA, 5.2.3/04

Report:

2009, Acute Inhalation Toxicity Study of Glyphosate TC in Rats.

Data owner: Helm AG

Report No.: 23911

Date: 2009-11-09

not published

ASB2012-11409

Guidelines:

EC method B.2. (92/69/EEC), OECD 403 and OPPTS 870.1300.

Deviations:

There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study.

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	2010-08-01
Test animals:	
Species:	Rat albino
Strain / Stock:	CD / CrI:CD(SD)
Source:	
Age:	Males: 52 days Females: 66 days
Sex:	5 male and 5 female
Weight at dosing:	Males: 240 - 267 g Females: 209 - 216 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), <i>ad libitum</i> except for approx 16 h before dosing
Water:	Tap water, <i>ad libitum</i>
Housing:	Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle

Study design and methods

In life dates: 2009-02-04 to 2009-07-30

Animal assignment and treatment:

The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was micronized before administration and the dust was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15 minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. A laser measured the size of the individual particles or individual aerosol drops. Animals were exposed four 4 hours to an actual concentration of 5.12 mg/L air (determined by HPLC).

After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion

Dust concentration and particle size distribution

The actual dust concentration of 5.12 mg glyphosate TC/L air was measured at the animals' nose and was determined by HPLC.

Laser measurement revealed the following particle size distribution during the exposure:

Diameter	Actual concentration 5.12 mg/L air
d _[10]	5.64 µm
d _[50]	6.62 µm
d _[90]	8.10 µm
[xx] = percentage of cumulative particle size distribution	

No finer dust concentration of the test item could be generated.

Mortality: There were no mortalities during the study.

Clinical observations: A 4-hour exposure to glyphosate TC at the concentration of 5.12 mg/L revealed slight dyspnoea and ataxia in all 5 of 5 male and 5 of 5 female animals immediately until 60 minutes after the end of exposure.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The acute inhalation LC₅₀ of the test material (glyphosate TC) in rats (males and females combined) was estimated to be greater than 5.12 mg/L air/4 hours. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute inhalation toxicity.

Comment by RMS:

Under the present conditions the study is considered acceptable and the acute inhalation LC₅₀ > 5 mg/L air/4 hours is agreed.

Reference: IIA 5.2.3/05

Report: [REDACTED] 2010 Acute Inhalation Toxicity Study of Glyphosate TC in Rats.

Data owner: Helm AG

Report No.: 24875

Date: 2010-06-03, not published

ASB2010-11410

Guidelines: EC method B.2. (92/69/EEC), OECD 403 and OPPTS 870.1300.

Deviations: There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study.

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	96.4%
Stability of test compound:	May 2011
Test animals:	
Species:	Rat albino
Strain / Stock:	CD / CrI:CD(SD)
Source:	
Age:	Males: approx. 7 weeks Females: approx. 9 weeks
Sex:	5 male and 5 female
Weight at dosing:	Males: 270 - 282 g Females: 220 - 251 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), <i>ad libitum</i> except for approx. 16 h before dosing
Water:	Tap water, <i>ad libitum</i>
Housing:	Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle

Study design and methods

In life dates: 2009-10-15 to 2010-02-18

Animal assignment and treatment:

The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15 minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. Animals were exposed four 4 hours to an actual concentration of 5.02 mg/L air (determined by HPLC). A laser measured the size of the individual particles or individual aerosol drops. The particle size distribution for the estimation of the Mass Median Aerodynamic Diameter (MMAD) was carried out twice during the exposure period using a cascade impactor. The median particle size distribution of the test item was determined with a Malvern Sizer.

After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once

daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion

Dust concentration and particle size distribution

Dust concentration and particle size distribution

The actual dust concentration of 5.02 mg glyphosate TC/L air was measured at the animals' nose and was determined by HPLC. The mean actual exposure concentration of glyphosate TC was as follows:

Table B.6.2-13: Details of test atmosphere

Actual concentration (HPLC) [mg/L air]	Actual concentration (gravimetric method) [mg/L air]	MMAD [μm]	Respirable amount particle size ≤4 μm	
			[mg/L air]	[%]
5.02	4.99	4.197 ± 2.64	1.03	20.5

No smaller MMAD could be obtained with the test item and no higher fraction of respirable particles could be obtained.

Laser measurement revealed the following particle size distribution during the exposure:

Diameter	Actual concentration 5.02 mg/L air
d _[10]	12.51 μm
d _[50]	37.15 μm
d _[90]	86.42 μm
[xx] = percentage of cumulative particle size distribution	

The particle size distribution of the delivered test item was d[50] = 14.5 μm.

Mortality: There were no mortalities during the study.

Clinical observations: A 4-hour exposure to glyphosate TC at the concentration of 5.02 mg/L revealed slight ataxia, slight tremor and slight dyspnoea immediately until 3 hours after the end of exposure.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The acute inhalation LC₅₀ of the test material (glyphosate TC) in rats (males and females combined) was estimated to be greater than 5.02 mg/L air/4 hours. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute inhalation toxicity.

Comment by RMS:

Under the present conditions the study is considered acceptable and the acute inhalation LC₅₀ > 5 mg/L air/4 hours is agreed.

Reference: IIA, 5.2.3/06
Report: [REDACTED] 2009
Glyphosate – Acute Inhalation Toxicity Study in Rats.
[REDACTED]
Data owner: Helm AG
Report No.: 12107-08
Date: 2009-03-09, not published
ASB2012-11411
Guidelines: US EPA OPPTS 870.1300.
Deviations: Humidity was in the range of 33-89 % instead of 30-70 %. Female weight was outside the protocol range. These minor deviations did not affect the study outcome.
GLP: yes
Acceptability: Please see comment by RMS.

Materials and methods

Test material: Glyphosate
Identification: Glyphosate Tech Grade Mixed 5-Batch
Description: White powder
Lot/Batch #: 080704-1 thru 5
Purity: 96.71 %
Stability of test compound: No data given in the report.
Test animals:
Species: Rat albino
Strain / Stock: Sprague-Dawley
Source: [REDACTED]
Age: Approx. 7-8 weeks
Sex: 5 male and 5 female
Weight at dosing: Males: 262 - 289 g
Females: 172 - 191 g
Acclimation period: 5 days
Diet/Food: Formulab #5008 (PMI Feeds Inc.), *ad libitum* except during the exposure period
Water: Tap water, *ad libitum* except during the exposure period
Housing: Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30 - 70 %
Air changes: 10 - 12/hour
12-hour light/dark cycle

Study design and methods

In life dates: 2008-11-07 to 2008-11-21

Animal assignment and treatment:

The study was carried out using a 500 L nose-only stainless steel, dynamic flow inhalation chamber with 25 ports in 5 rows. Polycarbonate tubes were inserted into 10 designated individual ports. The test substance was ground for 10 hours and dried prior to exposure. The aerosol was generated from the undiluted test substance by a Venturi Aspirator and sprayed directly into the exposure chamber. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber. Animals were exposed to the aerosol for a period of 4 hours. The dust concentration in the inhalation chamber was determined gravimetrically twice per hour and nominally at the end of the exposure. Particle size, taken from the breathing zone of the animals, was determined twice during the exposure using a cascade impactor, and the mass median aerodynamic diameter (MMAD) and particle size distribution were calculated.

Observations for mortality and signs of pharmacological and/or toxicological effects were made frequently on the day of exposure and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to the inhalation exposure and on Days 7 and 14. On Day 14 after completion of exposure, all animals were euthanized by an intraperitoneal injection, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion

Dust concentration and particle size distribution: The exposure concentration was determined to be 2.24 mg/L with an average MMAD of 2.6 µm.

Mortality: There were no mortalities during the study.

Clinical observations: The only prominent in-life observations were piloerection and activity decrease. Animals were asymptomatic by Day 4.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The acute inhalation LC_{50} of the test material (glyphosate) in rats (males and females combined) was estimated to be greater than 2.24 mg/L air/4 hours.

Comment by RMS:

The study is considered acceptable and the acute inhalation $LC_{50} > 2.24$ mg/L air/4 hours is agreed.

Reference:

Report:

IIA, 5.2.3/07

██████████ 2005,

Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats – Limit Test.

██
Data owner: Helm AG

Report No.: 15276

Date: 2005-04-04, not published

ASB2012-11412

Guidelines:

OPPTS 870.1300 (1998), OECD 403 and JMAFF 59 NohSan No. 4200 (1985).

Deviations:

There were no deviations from the Study Plan.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registrants must not use data for the purposes of this document.

GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate acid technical
 Identification: Glyphosate acid technical
 Description: White crystalline powder
 Lot/Batch #: 040205
 Purity: 97.23 %
 Stability of test compound: Test substance was expected to be stable for the duration of testing.
 Test animals:
 Species: Rat albino
 Strain / Stock: Sprague-Dawley derived
 Source: XXXXXXXXXX
 Age: 9-10 weeks
 Sex: 5 male and 5 female
 Weight at dosing: Males: 280 - 318 g
 Females: 205 - 224 g
 Acclimation period: 13 days
 Diet/Food: Purina Rodent Chow #5012, *ad libitum*
 Water: Filtered tap water, *ad libitum*
 Housing: Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
 Environmental conditions: Temperature: 19-23 °C
 12-hour light/dark cycle

Study design and methods:

In life dates: 2003-05-17 to 2003-05-24

Animal assignment and treatment:

The study was carried out using a nose-only inhalation chamber with an internal volume of approximately 6.7 L and approximately 283 air changes per hour during the study. Animals were individually housed in polycarbonate holding tubes. The test item was micronized before administration and aerosolized using a dust generator which was directly connected to the inhalation chamber. Gravimetric samples were withdrawn at 6 intervals from the breathing zone of the animals to gravimetrically determine the dust concentration in the inhalation chamber. Particle size distribution of the test atmosphere was determined with an Andersen Cascade Impactor. Samples were withdrawn from the breathing zone of the animals at two intervals. Animals were exposed four 4 hours and 1 minute to an actual concentration of 2.04 mg/L air (determined gravimetrically).

Observations for mortality and clinical/behavioural signs of toxicity were made upon removal from the exposure chamber and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to test substance exposure and on Days 7 and 14.

On Day 14 after dosing, each animal was euthanized by an overdose of CO₂. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Results and discussion

Dust concentration and particle size distribution: The gravimetric and nominal chamber concentrations were 2.04 and 8.99 mg/L, respectively. the mass median aerodynamic diameter was estimated to be 2.5 µm based on the particle size distribution as measured with an Andersen Cascade Impactor.

Mortality: There were no mortalities during the study.

Clinical observations: All animals appeared active and healthy upon removal from the exposure chamber and over the entire 14-day observation period.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers:

The acute inhalation LC₅₀ of the test material (glyphosate acid technical) in rats (males and females combined) was estimated to be greater than 2.04 mg/L air/4 hours.

Comment by RMS:

The study is considered acceptable and the acute inhalation LC₅₀ > 2.04 mg/L air/4 hours is agreed.

Reference:

IIA, 5.2.3/08

Report:

2008

Acute Inhalation Toxicity Test of Glyphosate Technical in Rats (*Rattus norvegicus*).

Data owner: Helm AG

Report No.: RF-3996.309.377.07

Date: 2008-09-11, not published

ASB2012-11413

Guidelines:

OECD guideline 403

Deviations:

The experimental phase initiation and conclusion dates were updated. This deviation did not affect the study outcome.

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Glyphosate Technical

Identification:

Glyphosate Technical

Description:

Solid

Lot/Batch #:

20070606

Purity:

98.05 %

Stability of test compound:

No data given in the report.

Test animals:

Species: Rat albino (*Rattus norvegicus*)
 Strain / Stock: Wistar Hannover
 Source: [REDACTED]
 Age: Males: 9 weeks
 Females: 11 weeks
 Sex: 5 males and 5 females
 Weight at dosing: Males: 262 - 291 g
 Females: 178 - 208
 Acclimation period: 9 days
 Diet/Food: Nuvilab CR-1 pellet diet type for rodents (Nuvital
 Nutrients Ltda.), *ad libitum*
 Water: Potable drinking water, *ad libitum*
 Housing: Polypropylene rodents cages with autoclaved wood
 shavings and stainless steel mesh lids containing five rats
 of each sex per cage.
 Environmental conditions: Temperature: 19-25 °C
 Humidity: 30 - 70 %
 Air changes: 10-15/hour
 12-hour light/dark cycle

Study design and methods:

In life dates: 2008-06-06 to 2008-06-20

Animal assignment and treatment:

The study was carried out using an inhalation chamber with a nose-only exposure of the animals. The test item was aerosolised. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber. Animals were exposed to the aerosol at the maximum attainable concentration (5.211 mg/L) for a period of 4 hours. The actual concentration in the inhalation chamber was determined gravimetrically by taking eight equally time-spaced air samples from the breathing zone. Aerodynamic particle size distribution was determined two times using a Seven Stage Cascade Impactor.

After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made right after the exposure, and thereafter each working day. On Day 14 after completion of exposure, all animals were euthanised in a carbon dioxide chamber, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion

Dust concentration and particle size distribution

The mean actual concentration was 5.211 mg/L. The actual concentration of the test item in each sample was within the ± 15 % interval from the mean actual concentration, indicating that the test atmosphere was held stable over the 4-hour exposure period.

Analysis of the particle size distribution of samples from the breathing zone indicates that 4.92 to 5.15 % of the mass collected from the aerosol were within the respirable size range. The MMAD ranged from 18.555 to 19.901 μm and the geometric standard deviation (GSD) ranged from 2.869 to 2.914.

Mortality: There were no mortalities during the study.

Clinical observations: Clinical signs observed during the 14-day observation period included wheeze and dyspnoea. These acute respiratory signs started within the first day and reverted within the fourth day of the observation period.

Body weight: The mean body weight increased for both sexes, except for the males on the first post-exposure day. All animals exceeded their initial body weight by the conclusion of the experimental phase.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The acute inhalation LC₅₀ of the test material (glyphosate Technical) in rats (males and females combined) was estimated to be greater than 5.211 mg/L air/4 hours. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical is not to be classified for acute inhalation toxicity.

Comment by RMS:

The study is considered not acceptable: The MMAD ranged from 18.555 to 19.901 µm, which is far above what is recommended in OECD 403 (MMAD ranging from 1 to 4 µm) and therefore, only 4.72 to 5.15 % of the particles were within the respirable size range. No explanation was given by the author why the standard could not be achieved.

Reference:

IIA, 5.2.3/09

Report:

2007, Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats

Data owner: Nufarm

Report No.: B02327

Date: 2007-04-02, unpublished

ASB2012-11414

Guidelines:

European Communities, Directive 92/69/EEC, Part B.2 "Acute Toxicity (Inhalation)", published December 29, 1992.

- OECD Guidelines for Testing of Chemicals, Section 4, No. 403: "Acute Inhalation Toxicity", adopted May 12, 1981.

- U.S. Environmental Protection Agency, Health Effects Test Guidelines OPPTS 870.1300, Acute Inhalation Toxicity, August 1998.

- Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute Inhalation Toxicity Studies Guideline 2-1-3. Notification 12 NohSan

No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005.

English translation by

ACIS on 17 Oct 2005

Deviations:

The following, minor deviations from the study plan were considered not to have compromised the quality, integrity or outcome of the study.

- The reference to the JMAFF inhalation test guideline was altered on request of the Sponsor.
However, this did not affect the contents of the JMAFF inhalation test guideline.
- In the animal room, on brief occasions (for a total of less than 2 hours) the relative humidity was slightly higher than the upper limit of the target range given in the study plan.
- On the day of inhalation exposure (test day 1), the total aerosol generation period lasted 4 hours and 30 minutes, because a test aerosol was generated also for 30 minutes prior to the beginning of the exposure. This 30-minute pre-exposure aerosol generation period was used for fine-tuning of the settings of the aerosol generation and exposure system for the inhalation exposure. Consequently the nominal test atmosphere concentration was determined for the total of 4 hours and 30 minutes of aerosol generation (30 min pre-exposure aerosol generation without animals being present plus 4 h inhalation exposure of the animals).

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:

Identification:

Glyphosate Technical (NUP 05068)

Description:

White powder

Lot/Batch #:

200609062

Purity:

95.1 %

Stability of test compound:

Stable under storage conditions.

Vehicle and/or positive control:

None

Test animals:

Species:

Rat

Strain:

HanRcc:WIST (SPF)

Source:

[REDACTED]

Age:

Males: 9 weeks

Females: 10 weeks

Sex:

Male / Female

Weight at dosing:

Males 241.6 – 257.4 g

Females 200.6 – 219.8g

Acclimation period:

5 days

Diet/Food:

Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/ Switzerland) ad libitum

Water:

Tap water, ad libitum

Housing:

During acclimatization in groups of five per sex in Makrolon type-4 cages with standard softwood bedding.

Environmental conditions: Temperature: 19-20 °C
 Humidity: 35-78 %
 Air changes: 10 - 15/hour
 12-hour light/dark cycle

Study design and methods

In life dates: 14-DEC-2006 to 28-DEC-2006

Animal assignment and treatment:

A dust aerosol was generated from the milled and pre-dried test item using a rotating brush aerosol generator (CR 3020, CR Équipements SA, CH-1295 Tannay, Switzerland) connected to a micronising jet mill. No extra diluent air was added. The generated aerosol was discharged into the exposure chamber through a 63Ni charge neutraliser. The achieved mean aerosol concentration of 3.252 mg/L air administered for 4 hours was considered to represent the highest technically achievable concentration suitable for acute inhalation toxicity testing in rodents. An increase in aerosol concentration by an increased supply of test item to the rotating brush of the aerosol generator would have led to complete blockage of the rotating brush (which had happened in a pre-study technical trial not performed under GLP), and consequently to complete blockage of the aerosol generation and exposure system. Two generator cylinders containing test item were needed in order to generate the highest technically achievable aerosol concentration over a 4-hour and 30-minute aerosol generation period.

The test atmosphere enters the top under slight positive pressure and is distributed to the entrance of each feed tube. It is then delivered through these tubes to the animal's nose. The inhalation exposure system is located inside a ducted extraction cabinet. Test atmosphere samples for the gravimetric measurements of the test item concentration and particle size distribution, and for the measurement of temperature, relative humidity and oxygen concentration, were collected directly from the feed tube in the breathing zone of the animals, at an empty port of the exposure chamber delivering "fresh" test item to the animal's nose. This approach was chosen in order to obtain representative samples of what was delivered to the animals.

The particle size distribution was determined twice during the exposure using a Mercer 7 stage cascade impactor (Model 02-130, In-Tox Products Inc., Albuquerque, New Mexico, U.S.A.).

Representative samples of the test atmosphere were drawn through the impactor with a flow rate of 1.0 L/min and the particles deposited according to their aerodynamic size onto stainless steel

slips and the final filter stage (Type HVLP, Polyvinylidenedifluoride membrane, pore size 0.45 µm), on each stage of the impactor. To obtain the mass deposited on each stage of the impactor, the

steel slips and the final filter stage were carefully weighed before and after sampling using a Mettler MX5 analytical balance (Mettler AG, CH-8604 Volketswil, Switzerland). The total mass (µg) deposited in the impactor was then calculated by adding together the mass deposited on each of the stainless steel slips and the final filter stage. As the Effective Cut-off Diameters (ECD) represent the lower size limit of the particles collected on each stage, the cumulative percent less than the indicated size was tabulated as a function of the ECDB. This data was used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) using Microsoft Excel software. The target range for the MMAD was 1 to 4 µm, and was achieved.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The following clinical signs were recorded during and/or after the inhalation exposure, whereby the whole range of the stated severity grades was not necessarily recorded in each affected animal: Salivation, moderate in degree, and deep respiration in two male animals (nos. 3 & 5), and breath sounds [rales], slight to marked in degree, in three male (nos. 1, 3 & 5) and two female animals (nos. 6 & 10).

The findings of salivation and deep respiration were seen at approximately 3 and 4 hours after exposure start, when the animals were restrained in the exposure tubes. Deep respiration was still evident one hour afterwards, at approximately one hour after the end of the exposure period. Breath sounds [rales] were only noticed at approximately one hour after the end of the exposure period and on the day afterwards (test day 2) after the animals had returned to their housing cages. By two days after the inhalation exposure (test day 3) all clinical signs had cleared, and all animals remained free from clinical signs until the scheduled necropsy day (test day 15).

Body weight: Losses in body weight were evident in three of five male animals (mean loss in the affected males –3.0 %) and three of five female animals (mean loss in the affected females –2.1 %), and retardation in body weight gain in one other male animal (+0.8 % weight gain) over the first three days following the inhalation exposure (test days 1 to 4). The effects on body weight were only transient and were followed by normal body weight gain in all animals.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The LC_{50} of glyphosate technical (NUP 05068) for acute 4-hour inhalation toxicity in male and female rats observed for a period of 15 days, was estimated to be greater than 3.252 mg/L air (gravimetrically determined mean aerosol concentration). This concentration was considered to represent the highest technically achievable aerosol concentration suitable for acute inhalation toxicity testing in rodents.

Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

Comment by RMS:

The study is considered acceptable and the acute inhalation $LC_{50} > 3.252$ mg/L air/4 hours is agreed.

Reference:

IIA, 5.2.3/10

Report:

1996, Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats

Data owner: Syngenta

Report No.: /P/4882

Date: 1996-04-29, not published

TOX2000-1984

Guidelines:

OECD 403 (1981): OPPTS 870.1300 (1998): 92/69/EEC B.2 (1992)
+ amendment 93/21/EEC (1993)

Deviations:

None

GLP: yes
Acceptability: Please see comment by RMS

Table B.6.2-14: Details of test atmosphere

Target concentration mg/L	Achieved particulate concentration mg/L	MMAD* μm	GSD ⁺
2	2.47- \pm 0.15	3.57, 3.03	1.94, 1.90
5	4.43 \pm 1.297	2.91, 3.41	1.74, 2.04

* Mass Median Aerodynamic Diameter (μm)

+ Geometric Standard Deviation

Two males and two females exposed to 4.43 mg glyphosate acid/L were found dead or were terminated *in extremis* during the observation period, the remaining animals in this group survived until scheduled termination. Clinical signs indicative of moderate toxicity were seen in this group. All surviving animals had regained their initial bodyweight by the end of the study.

Similar but less severe clinical signs were seen in animals exposed to 2.47 mg/L, all animals survived and showed complete recovery by the end of the study. All animals exposed to 2.47 mg/L survived to scheduled termination

It was concluded that the acute inhalation LC₅₀ of glyphosate acid exceeded 2.47 mg/L for male rats and exceeded 4.43 mg/L for female rats

Materials and methods

Test material:

Identification: Glyphosate acid

Description: Technical; white solid

Lot/Batch #: P25

Purity: 95.6 % w/w

Stability of test compound: Confirmed by Sponsor

Vehicle and/
or positive control: None

Test animals:

Species: Rat

Strain: Alpk:APfSD

Source:

Age: Young adult; 9 - 12 weeks old at delivery

Sex: Males and females

Weight at dosing: 243-365 g (males); 210-247 g (females) at the start of exposure

Acclimation period: At least five days

Diet/Food: PCD diet (Special Diet Services Limited, Witham, Essex, UK) *ad libitum* except during exposure.

Water: Mains water *ad libitum* except during exposure.

Housing: 5 per cage, sexes separately, except during exposure, in rat racks suitable for animals of the strain and weight range expected during the study.

Environmental conditions: Temperature: 19-20 °C
Humidity: 40-70 %
Air changes: at least 15/hour
Photoperiod: 12-hour light/dark cycle

Study design and methods

In-life dates: Start: 22 November 1995 End: 11 March 1996

Exposure conditions: Trial generations were carried out prior to the start of the study in order to determine the appropriate generation system and conditions, to determine the appropriate target concentration that could be achieved, or if not, what was the maximum stable attainable concentration, to obtain data on the aerodynamic particle size of the atmosphere generated, to determine an appropriate method of analysis of glyphosate acid. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of glyphosate acid.

Animal assignment and treatment: The study consisted of two main study groups of 5 rats/sex/group exposed nose-only for a single four-hour period to glyphosate acid at target particulate concentrations of 5 mg/L and 2 mg/L. Prior to the start of the study the rats were examined to ensure that they were physically normal and exhibited normal activity. During exposure they were observed frequently and, at the end of the 4-hour exposure period, each rat was given a detailed clinical examination. They were also subjected to detailed clinical observations, daily during a 14-day observation period. The bodyweight of each rat was recorded on day -1, 1, 8 and prior to termination on day 15. All rats were killed on day 15 and subjected to a gross examination *post mortem* involving external observation and careful internal examination of all thoracic and abdominal viscera.

Generation of the test atmosphere / chamber description: Before exposure of the test animals, the atmosphere was shown to have been acceptably stable. The test atmosphere was generated using a modified Wright's dust-feed mechanism. Clean, dry air was passed through the dust feed at a nominal flow rate of 2.5 L/minute (at normal temperature and pressure) and carried the atmosphere to the exposure chamber, having an internal volume of 27.6 litres. Since diluting air was not employed, the flow rate through the exposure chamber was the same as that employed in the generation of the test atmosphere. Air flows were monitored and recorded at approximately 30 minute intervals using variable area flow-meters and were altered as necessary to maintain target concentration. Animals were exposed nose-only to the atmosphere. They were restrained in polycarbonate tubes (Battelle, Switzerland), which were inserted into the Perspex exposure chamber. The chamber was covered with an aluminium cone and stood on an aluminium base.

Test atmosphere concentration: The particulate concentration of the test atmosphere, close to the animals' breathing zone, was measured gravimetrically at frequent intervals during the exposure period. This was done by drawing the test atmosphere, at a known flow rate, for a known time, through a 25 mm diameter, polyvinyl chloride (PVC) GLA 5000 filter housed in a Delrin open-faced filter holder. The filter was weighed before and after the sample was taken. The concentration was calculated as follows:

Concentration (mg/L) = $\frac{\text{post wt (mg)} - \text{pre wt (mg)}}{\text{volume (L)}}$

time (minutes) x airflow
(L/minute)

Pre wt = weight of filter prior to sampling

Post wt = weight of filter after sampling

Particle size determination: The aerodynamic particle size distribution of the test atmosphere was measured twice during the exposure period, using a Marple Cascade Impactor, which aerodynamically separates airborne particles into pre-determined size ranges. Using a microcomputer, the data were transformed using a log/probit transform and a linear regression derived from the cumulative data. The linear regression line was then used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

Table B.6.2-15: Summary of acute study test atmosphere characteristics

Parameter	Target concentration 5 mg/L				Target concentration 2 mg/L			
Measured particulate concentration	4.43 ± 1.30 mg/L				2.47 ± 0.15 mg/L			
% total particulate	96.9 ± 4.2				98.5 ± 7.7			
Analysed concentration of glyphosate acid(mg/L)	Mean 4.27 ± 1.15				Mean 2.43 ± 0.19			
Particle size MMAD; GSD	2.91, 3.41µm; 1.74, 2.04				3.57, 3.03µm; 1.94, 1.90			
Size range (µm)	% by weight in range				% by weight in range			
	Run 1 (1hr 35min into exposure)		Run 2 (3hr 29min into exposure)		Run 1 (54min into exposure)		Run 2 (2hr 59min into exposure)	
	Analysed	Gravimetric	Analysed	Gravimetric	Analysed	Gravimetric	Analysed	Gravimetric
Particles > 9.8 µm (% w/w)	0.9	0.7	5.9	4.3	3.1	3.1	2.0	2.0
Particles 9.8-6.0 µm (% w/w)	21.1	20.6	26.3	23.8	20.9	23.0	16.7	16.4
Particles 6.0-3.5 µm (% w/w)	34.4	35.5	34.3	31.3	47.9	46.4	37.7	36.8
Particles 3.5-1.55 µm (% w/w)	32.1	32.3	21.3	28.0	19.5	18.8	38.0	36.9
Particles 1.55-0.93 µm (% w/w)	7.5	7.6	9.1	8.6	5.9	5.4	3.6	4.0
Particles 0.93-0.52 µm (% w/w)	2.2	2.3	2.7	2.3	1.9	2.0	1.3	1.6
Particles ≤0.52 µm (% w/w)	1.5	1.0	1.3	1.8	0.8	1.3	0.8	2.4
Flow rate (whole system)	2.5 L/min							
Temperature	14.7 – 21.7 °C							
Humidity	25 - 65 %							

- Percentages are calculated as follows:

Gravimetric: $\frac{\text{weight trapped at each size range} \times 100}{\text{Total weight trapped}}$

Statistics: The acute inhalation LC₅₀ was estimated.

Results and discussion

Mortality: Two males and two females exposed to 4.43 mg/L were found dead or were terminated in extremis on days 5, 6 or 9 of the study, the remaining animals in this group survived until scheduled termination.

There were no mortalities at 2.47 mg/L.

Table B.6.2-16: Mortality / animals treated

Target exposure concentration mg/L	Day number	Cumulative mortality (Number dead / total)		
		Males	Females	Combined
5	5	1/5	0/5	1/10
	6	2/5	1/5	2/10
	9	2/5	2/5	3/10
	Day 14	2/5	2/5	4/10
2	Day 14	0/5	0/5	0/10

Clinical observations: Abnormalities generally associated with restraint (wet fur) were seen in all animals during exposure. Clinical changes seen were salivation, irregular breathing and auditory hypoaesthesia, these effects were considered to be related to treatment.

Immediately after exposure, abnormalities generally associated with restraint (hunched posture, piloerection and wet fur) were seen in both males and females. At an exposure concentration of 4.43 mg/L the clinical abnormalities seen in both sexes included breathing irregularities, reduced righting reflex, shaking, splayed gait and were considered to be indicative of moderate toxicity.

At an exposure concentration of 2.47 mg/L the number of adverse clinical changes observed was reduced in both sexes. Those abnormalities observed were similar to those seen in animals exposed to 4.43 mg/L glyphosate acid.

The clinical condition of most animals appeared to have improved by day 5 of the study, with the exception of 2 males and 2 females exposed to 4.43 mg/L. There was generally an improvement in clinical condition during the remainder of the study.

Bodyweight: Animals showed a treatment related reduction in bodyweight. At an exposure concentration of 4.43 mg/L all animals had exceeded their initial bodyweight by the end of the study. At an exposure concentration of 2.47 mg/L all animals had exceeded their initial weight by day 8 of the study.

Necropsy: In the animals exposed to 4.43 mg/L that died or were killed prior to termination, the two males found dead had dark lungs (probably a result of agonal congestion), the lungs of the females were normal.

At scheduled termination, the lungs of rats exposed to 4.43 mg/L were normal. One female exposed to 2.47 mg/L had red spots on the lungs and another female had dark lungs. These findings are considered to be incidental to treatment. Changes at necropsy in a variety of tissues in males exposed to 2.47 mg/L were of low incidence and were considered to be unrelated to treatment.

Conclusion by the Notifiers:

It was concluded that the acute inhalation LC_{50} of glyphosate acid exceeded 2.47 and exceeded 4.43 mg/L for male and female rats.

Comment by RMS:

The study is considered acceptable and the acute inhalation $LC_{50} > 4.43$ mg/L air/4 hours is agreed.

Reference: IIA, 5.2.3/11
Report: [REDACTED] 2011,
 Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat
 [REDACTED]
 Data owner: Syngenta
 Report No.: 11/054-004P
 Date: 2011-06-06
 GLP:
 not published
 ASB2012-11415
Guidelines: OECD 403 (1981): OPPTS 870.1300 (1998): 440.2008 B.2 (2008)
Deviations: None
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:
 Identification: Glyphosate technical
 Description: Technical; dry white powder
 Lot/Batch #: 614034 (20100609 Milled)
 Purity: 96.9 % w/w glyphosate technical
 Stability of test compound: Stable under storage conditions (room temperature range <30 °C), recertification date end January 2014
 Vehicle and/or positive control: None
 Test animals:
 Species: Rat
 Strain: RjHan:WI
 Source: [REDACTED]
 Age: 8-10 weeks
 Sex: Males and females
 Weight at dosing: 229-386 g
 Acclimation period: At least five days
 Diet/Food: ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany *ad libitum* (except during exposure)
 Water: Tap water *ad libitum* (except during exposure)
 Housing: In groups of 5 (or 2 in the case of sighting exposure), by sex, in solid-floor cages (Type III) with stainless steel mesh lids and softwood flake bedding.

Environmental conditions: Temperature: 22 ± 3 °C
 Humidity: 30-70 %
 Air changes: 15-20 air changes per hour
 Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 14 April 2011 End: 28 April 2011

Exposure conditions: Prior to animal exposures, test material atmospheres were generated within the exposure chamber. During these technical trials, air-flow settings and test material input rates were adjusted to achieve the required atmospheric characteristics. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of Glyphosate Technical.

Exposure system: The animals were exposed, nose-only, to an atmosphere of the test item using a TSE Rodent Exposure System (TSE Systems GmbH, Bad Homburg, Germany). This system comprises of 2, concentric anodised aluminium chambers and a computer control system incorporating pressure detectors and mass flow controllers.

Fresh aerosol from the generation system was constantly supplied to the inner plenum (distribution chamber) of the exposure system from where, under positive pressure, it was distributed to the individual exposure ports. The animals were held in polycarbonate restraint tubes located around the chamber which allowed only the animal's nares to enter the exposure port. After passing through the animal's breathing zone, used aerosol entered the outer cylinder from where it was exhausted through a suitable filter system. Atmosphere generation was therefore dynamic.

Airflows and relative pressures within the system were constantly monitored and controlled by the computer system thus ensuring a uniform distribution and constant flow of fresh aerosol to each exposure port (breathing zone). The flow of air through each port was at least 0.7 L/min. This flow rate was considered adequate to minimise re-breathing of the test atmosphere as it is about twice the respiratory minute volume of a rat.

Homogeneity of the test atmosphere within the test chamber and amongst the exposure ports was not specifically determined during this study. However, chambers of this design have been fully validated and have shown to produce evenly distributed atmospheres in the animals' breathing zones.

Exposure procedure: Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber. Only the nose of each animal was exposed to the test atmosphere. Following an equilibration period of at least the theoretical chamber equilibration time (T99), a group of 10 rats (5 male and 5 female) was exposed to a target atmosphere concentration 5 mg/L for a period of at least 4 hours.

Generation of the test atmosphere / chamber description: The test item was aerosolised using a rotating brush powder disperser (Palas GmbH, Karlsruhe, Germany) located at the top of the exposure chamber. Compressed air was supplied by means of an oil-free compressor and passed through a suitable filter system prior to introduction to the dust generator.

Test atmosphere concentration: The test atmosphere was sampled at regular intervals during each exposure period. Samples were taken from an unoccupied exposure port (representing the animal's breathing zone) by pulling a suitable, known volume of test atmosphere through weighed GF10 glass fibre filters. The difference in the pre and post sampling weights, divided by the volume of atmosphere sampled, was equal to the actual achieved test atmosphere concentration.

The nominal concentration was calculated by dividing the mass of test material disseminated into the chamber by the total volume of air that through the chamber during the same period.

Particle size determination: The particle size of the test atmosphere was determined three times during the exposure period using a 7-stage impactor of Mercer style (which employs an inertial separation technique to isolate particles in the discrete aerodynamic size ranges). Samples were taken from an unoccupied exposure port (representing the animal's breathing zone).

The collection substrates and the backup filter were weighed before and after sampling and the weight of test item, collected at each stage, calculated by this difference.

The total amount collected for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 0.55, 0.96, 1.55, 2.11, 3.56, 6.66 and 10.55 μm was calculated.

From these data, using software supplied with the impactor (TSE Systems GmbH, Bad Homburg, Germany), the Mass Median Aerodynamic Diameter (MMAD), and Geometric Standard Deviation were calculated. In addition, the proportion (%) of aerosol less than 4 μm (considered to be the inhalable portion) was determined.

Table B.6.2-17: Summary of main study test atmosphere characteristics

Parameter	Target concentration 5 mg/L	
Mean achieved concentration (mg/L)	5.04 \pm 0.17	
Nominal (mg/L)	7.71	
Particle size MMAD; GSD	3.65 μm ; 2.24	
Inhalable fraction (% < 4 μm)	54.4	
	% by weight in range #	
Size range (μm)	Total mass/stage (mg)	Cumulative mass (%)
<0.55	0.35	2.05
0.55 – 0.96	0.30	3.81
0.96 – 1.55	0.91	9.13
1.55 – 2.11	1.90	20.26
2.11 – 3.56	5.43	52.05
3.56 – 6.66	4.69	79.51
6.66 – 10.55	2.06	91.57
>10.55	0.44	100.00
T99 (Minimum Acceptable Equilibration Time)	1 minute	
Chamber volume (inner plenum)	3.85 L	
Air Flow In (Inner Plenum) (L/min)	20.0-20.6	
Air Flow Out (Inner Plenum) (L/min)	19.4 – 38.4	
Temperature	21.6 – 24.7 $^{\circ}\text{C}$	
Humidity	3.9 – 10.2 % (n = 3)	
Oxygen Concentration (%)	19.6 – 20.3	
Carbon Dioxide	0.1 – 0.8	

Sighting studies: Two sighting exposures using 2 male and 2 female rats were performed before the main study due to insufficient information about the test item's inhalation toxicity.

Animal assignment and treatment: Five male and 5 females were exposed to a target aerosol concentration of 5 mg/L glyphosate technical. The animals were exposed for 4 hours using a nose-only exposure system, followed by a 14 day observation period. The day of exposure was designated Day 0.

Animals were checked hourly during exposure, 1 hour after exposure and twice daily (early and late in the working day) during the 14 days of the observation period for morbidity and/or mortality. All animals were observed for clinical signs at hourly intervals during exposure, as soon as practically possible following removal from restraint at the end of exposure, 1 hour

after exposure and subsequently once daily for 14 days. The body weight of each rat was recorded prior to treatment on the day of exposure (day 0) and on Days 1, 3, 7 and 14.

At the end of the 14 day observation period, the animals were sacrificed by exsanguination under anaesthesia and a gross macroscopic examination was performed, which included a detailed examination of the abdominal and thoracic cavities. Special attention was given to the respiratory tract for macroscopic signs of irritancy or local toxicity.

Statistics: The acute inhalation LC_{50} was calculated from the mortality data.

Results and discussion

Mortality: One male rat died following a 4 hour exposure to 5.04 mg/L glyphosate technical.

Clinical observations: Wet fur and fur staining were commonly recorded on the day of and the day following exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be treatment related.

Significant clinical signs were recorded on day of exposure and the following day included laboured and noisy respiration, respiratory rate increased, gasping respiration, sneezing, decreased activity, thin body appearance (weak/wasted).

The majority of animals recovered from Day 3.

Body weight: Normal body weight gain was noted for all surviving animals from Day 1, with the exception of one male where a slight bodyweight loss was recorded during first week of the observation period.

Necropsy: There were no macroscopic abnormalities in animals surviving to scheduled termination. A specific cause of death was not determined for the single male that died in the main study.

Conclusion by the Notifiers:

Under the experimental conditions of this study, a single death occurred in a group of 10 rats exposed to a mean achieved atmosphere of 5.04 mg/L for 4 hours. The acute inhalation LC_{50} of glyphosate technical, in Wistar Kijang (WI) strain rats is considered to be greater than 5.04 mg/L.

Comment by RMS:

The study is considered acceptable and the acute inhalation $LC_{50} > 5.04$ mg/L air/4 hours is agreed.

Reference:

IIA, 5.2.3/12

Report:

1999, NUP5a99 62% glyphosate MUP: Acute inhalation toxicity study in rats – Limit test

Data owner: Nufarm

Study No.: 7909

Date: 1999-09-16, unpublished

ASB2012-11416

Guidelines:

Health Effects Test Guidelines, OPPTS 870.1300 (1998)

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Identification: NUP5a99 62 % glyphosate MUP

Description: clear viscous amber liquid

Lot/Batch #: Drum Sample E

Purity: 62 %

Stability of test compound: No data available

Vehicle and/
or positive control: None

Test animals:

Species: Rat

Strain: Sprague-Dawley derived, albino

Source:

Age: Not specified

Sex: 5 males and 5 females

Weight at dosing: males 224-256 grams and females 179-201 gramsg

Acclimation period: 10 days

Diet/Food: Purina Rodent Chow #5012

Water: Tap water, ad libitum

Housing: singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.

Environmental conditions: Temperature: 22-24 °C
Humidity: not specified
Air changes: not specified
12-hour light/dark cycle

Study design and methods

In life dates: August 6-20, 1999

Animal assignment and treatment:

Prior to initiation of the full inhalation study, pre-test trials were conducted to establish generation procedures for achieving as closely as possible the desired chamber concentration (2.0 mg/L) and desired particle size distribution (mass median aerodynamic diameter $\leq 4 \mu\text{m}$). The animals were placed in a rectangular whole body plexiglass chamber with a volume of 150 liters with prechamber operated under slight negative pressure, and were exposed to the test atmosphere for 4 hours and 15 minutes. The exposure period was extended beyond 4 hours to allow the chamber to reach equilibrium (T99). The times for 90 and 99 % equilibration of the chamber atmosphere were 7.5 and 15.1 minutes, respectively. The gravimetric and nominal chamber concentrations were 2.08 and 18.38 mg/L, respectively. The mass median aerodynamic diameter was estimated to be 2.6 microns based on the particle size distribution as measured with an Andersen Cascade Impactor. At the end of the exposure period, the generation was terminated and the chamber was operated for a further 15 minutes with clean air. At the end of this period the animals were removed from the chamber. Prior to being returned to their cages, excess test substance was removed from the fur of each animal.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: In-chamber animal observations included ocular and nasal discharge, hunched posture and hypoactivity. Apart from test substance noted on the fur, all animals recovered from the above symptoms upon removal from the exposure chamber and appeared active and healthy throughout the study.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers:

The single exposure acute inhalation LC_{50} of NUP5a99 62 % glyphosate MUP is >2.08 mg/L. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for this end point.

Comment by RMS:

The study is considered acceptable and the acute inhalation $LC_{50} > 2.08$ mg/L air/4 hours is agreed.

Reference:

IIA, 5.2.3/13

Report:

2004,
An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623

Data owner: Monsanto

Monsanto Report No.: SB-2003-116

Date: 2004-02-06, not published

ASB2012-11415

Guidelines:

EC method B2, OECD 403, EPA Health Effects Test Guidelines, OPPTS 870.1300, JMAFF 12 Nohsan No. 8147

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Identification:

MON 78623

Description:

Clear colourless liquid (pipet), light amber liquid (bulk)

Lot/Batch #:

GLP-0306-14124-F

Purity:

47.2 % glyphosate (57.8 % potassium salt of glyphosate)

Stability of test compound:

Expiry June, 2004

Vehicle and/
or positive control:

None

Test animals:

Species:

Rat

Strain:

Sprague Dawley

Source:	
Age:	8-9 weeks
Sex:	Males and females
Weight at dosing:	♂ 276 – 312 g; ♀ 182 – 210 g
Acclimation period:	At least 5 days
Diet/Food:	PMI Certified Rodent Chow #5002 (PMI Nutrition International), <i>ad libitum</i> (except during acclimatisation to the exposure tubes and during the exposure)
Water:	Tap water, <i>ad libitum</i> (except during acclimatisation to the exposure tubes and during the exposure)
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: 19 - 23 °C Humidity: 31 - 65 % Air changes: 10-15 per hour Light cycle: 12 hours light/dark cycle

Study design and methods

In life dates: 2003-10-29 to 2003-12-29

Test atmosphere generation:

The test aerosol was generated with a Master Flex Pump and Pump Head (7523-30 and 77200-60) and a Pistol Spraying System. Conditioned high pressure external air was used in generating the test atmosphere. The aerosol was blown through a 5 L Elutriator, the Multi-Stage 10 L nose-only inhalation chamber and then vented from the chamber to an air treatment system which consisted of a prefilter, a HEPA filter, a charcoal bed and a water scrubbing tower.

Exposure chamber conditions:

Air flow readings were recorded at the initiation of the T99 equilibration period, at approximately 30-minute intervals during each aerosol exposure and at the conclusion of the de-equilibration period. The aerosol concentration was measured at the beginning of each aerosol exposure (after equilibration), at approximate 30-minute intervals during the aerosol exposure, and at the conclusion of each aerosol exposure (before de-equilibration). Samples of the test article aerosol were collected in the inhalation chamber by gravimetric technique. Both gravimetric and analytical aerosol concentrations were determined. A 5 L sample of the aerosol was drawn from the breathing zone of the animals in the chamber through a preweighed glass fiber filter. For the analytical concentration, the gravimetrically obtained samples were analysed by liquid chromatography for the non-volatile glyphosate component of the test article. These analyses were performed in order to determine the analytical (actual) concentrations of the aerosol in the chamber for each sampling period. Chamber oxygen content was measured and recorded at approximate 30-minute intervals during each aerosol exposure using a GC-501 Oxygen Detector.

Particle size distribution:

The aerosol aerodynamic particle-size distribution was determined three times during each aerosol exposure using the ITP 7 Stage Cascade Impactor. Each stage of the impactor was fitted with a preweighed glass fiber filter. Five liters per minute of the chamber air were drawn through the impactor and the change in weight of each filter was then determined and recorded. The mean particle-size distribution was subsequently determined using an Excel

computer adaptation of the manual method. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and percentage of particles $\leq 4.0 \mu$ were then determined. The results were as follows:

Table B.6.2-18: Details of test atmosphere

Mean Achieved Actual Concentration (analytical method)	MMAD	GSD	Respirable Amount Particle Size $\leq 4 \mu\text{m}$
(mg/L)	(μm)		(%)
2.21	2.9	2.18	67
5.27	3.8	2.20	53

MMAD = mean mass median aerodynamic diameter

GSD = geometric standard deviation

Animal assignment and treatment:

The animals chosen for study use were randomly selected from healthy stock animals using a computerized random numbers table to avoid potential bias. On day 0, the animals chosen for the limit test were weighed, placed in a nose-only exposure tube and allowed to acclimate to the exposure tube for at least one hour. Animals that appeared to have been acclimated to the exposure tube (i.e., minimal struggling and no inversion) were considered to be acceptable. Animals that did not appear to acclimate to the exposure tube were not acceptable. All animals were removed from the exposure tubes and returned to their cages.

The acceptable animals were then placed in exposure tubes and the tubes inserted into the Multi-Stage 10 L nose-only inhalation chamber and the test article aerosolised at the following levels:

Table B.6.2-19: Dose Levels

Analytical Exposure Level (mg/L)	No. of Animals	
	Male	Female
2.21	5	5
5.27	5	5

Each aerosol exposure consisted of a 3-minute T99 equilibration period, a 240-minute exposure period and a 3-minute de-equilibration period equal to the T99 equilibration period. After each aerosol exposure, animals were removed from the exposure tubes and residual test article was removed from the animal's exterior surfaces (where practical) by wiping the haircoat with a towel. The animals were then returned to ad libitum feed and water.

The limit test animals were observed for clinical abnormalities during each aerosol exposure (no positive clinical observations were noted during either exposure), two times on study day 0 (post-exposure) and daily thereafter (days 1-14). Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanized and all study animals were subjected to gross necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The most notable clinical abnormalities observed for the 2.21 mg/L dose level included transient incidences of congested breathing and dark material around the facial area.

The most notable clinical abnormalities observed for the 5.27 mg/L dose level included transient incidences of congested breathing and few faeces.

Body weight: Body weight gain was noted for all animals for the 2.21 mg/L dose level.

For the 5.27 mg/L dose level, slight body weight loss was noted for two females during the day 0 to 7 body weight interval and for one female during the day 7 to 14 body weight interval. Body weight gain was noted for all other animals and all animals exceeded their initial body weight at study termination.

Necropsy: No gross internal findings were observed at necropsy for the 2.21 mg/L and 5.27 mg/L dose levels on study day 14.

Conclusion by the Notifiers

The acute inhalation LC_{50} for the test substance MON 78623 was estimated to be greater than 5.27 mg/L. According to EU and OECD Globally Harmonised System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity.

Comment by RMS:

The study is considered acceptable and the acute inhalation $LC_{50} > 5.25$ mg/L air/4 hours is agreed.

B.6.2.4 Skin irritation

According to the previous EU evaluation, glyphosate acid and its salts were considered non-irritant to intact skin and only slightly irritant to abraded skin. For the current re-evaluation, 13 additional studies with glyphosate acid on rabbits were submitted.

Table B.6.2-20: Summary of skin irritation studies with glyphosate acid

Reference (Data owner)		Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
Studies from the 2001 evaluation	Annex B- 5.2.5.1, Glyphosate Monograph [REDACTED] 1994 (Alkaloid) TOX9650145	Rabbit NZW	4	97.2	0.5 g; intact + abraded skin	Very slight irritation
	Annex B- 5.2.5.1, Glyphosate Monograph [REDACTED] 1991 (I. Pi. Ci.) TOX9551627	Rabbit NZW	3 ♂	98	0.5 g moistened with saline; intact skin	Very slight irritation

Reference (Data owner)		Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
	Annex B- 5.2.5.1, Glyphosate Monograph [REDACTED] 1991 (FSG) TOX9551092	Rabbit NZW	2 ♂, 1 ♀	96.8	0.5 g; intact skin	Non irritant
	Annex B- 5.2.5.1, Glyphosate Monograph [REDACTED] 1990 (AGC) TOX9551794	Rabbit NZW	3 ♂	98.1	0.5 g moistened with saline; intact + abraded skin	Non irritant
Studies from the 2001 evaluation	Annex B- 5.2.5.1, Glyphosate Monograph [REDACTED], 1989 (CHE) TOX9552333	Rabbit NZW	2 ♂, 4 ♀	98.6	0.5 g moistened with water; intact skin	Non irritant
Studies not reviewed in the 2001 evaluation	IIA 5.2.4/01 [REDACTED] 2007 (NUF) ASB2012- 11418	Rabbit NZW	1 ♂, 2 ♀	95.1	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
	IIA 5.2.4/02 [REDACTED] 2009 (HAG) ASB2012- 11419	Rabbit Himalaya n	3 ♂	96.4	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/03 [REDACTED], 1995 (ALS) ASB2012- 11420	Rabbit NZW	6 ♂	97.56	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
	IIA 5.2.4/04 [REDACTED] 2009 (HAG) ASB2012- 11421	Rabbit Himalaya n	3 ♂	98.8	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/05 [REDACTED] 2010 (HAG) ASB2012- 11422	Rabbit Himalaya n	3 ♂	97.3	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/06 [REDACTED] 2009 (HAG) ASB2012- 11423	Rabbit NZW	1 ♂, 2 ♀	96.4	0.5 g moistened with water; intact skin	Non irritant

Reference (Data owner)	Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
IIA 5.2.4/07 [REDACTED] 2005 (HAG) ASB2012- 11424	Rabbit, NZW	3 ♂	97.23	0.5 g moistened with water; intact skin	Slightly irritating
IIA 5.2.4/08 Canabrava [REDACTED] 2008 (HAG) ASB2012- 11425	Rabbit, NZW	3 ♀	98.05	0.5 g moistened with water; intact skin	Non irritant
IIA 5.2.4/09 [REDACTED] 1988 (MON) Z35394	Rabbit NZW	3 ♂, 3 ♀	97.76	0.5 g moistened with saline; intact skin; semi-occluded	Non irritant
IIA 5.2.4/10 [REDACTED] 1979 (MON) Z35544	Rabbit	3 ♂, 3 ♀	98.5	0.5 g moistened with water; intact skin; occluded	Primary dermal irritation index 0.1 (study not acceptable)
IIA 5.2.4/11 [REDACTED] 1996 (SYN) TOX2000-1985	Rabbit, NZW	6 ♀	95.6	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
IIA 5.2.4/12 [REDACTED] 2007 (SYN) ASB2012- 11426	Rabbit NZW	1 ♂, 2 ♀	96.1	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
IIA 5.2.4/13 [REDACTED] 2011 (SYN) ASB2012- 11427	Rabbit NZW	3 ♂	96.3	0.5 g moistened with water; intact skin	Mild Irritant (Primary dermal irritation index 0.11)

NZW = New Zealand White

Table 6.2-21: Summary of skin irritation studies with glyphosate salts

Reference (Data owner)	Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
Annex B 5.2.5.4 Glyphosate Monograph [REDACTED] 1994 (MON / CHE) TOX9552335	Rabbit NZW	1 ♂, 5 ♀	IPA 62%	0.5 mL (pure)	Slight irritation

Reference (Data owner)	Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
Annex B- 5.2.5.1, Glyphosate Monograph [REDACTED] 1994 (Herbex) TOX9500248	Rabbit NZW	2 ♂, 1 ♀	IPA 360 g/L	0.5 mL; intact skin	Non irritant
Annex B- 5.2.5.1, Glyphosate Monograph [REDACTED] 1989 (I.Pi.Ci.) TOX9551628	Rabbit NZW	3 ♂, 3 ♀	IPA 62	0.5 mL (pure); ♂: intact skin; ♀: abraded skin	Slight irritant
Annex B- 5.2.5.1, Glyphosate Monograph [REDACTED] 1987 (MON/CHE) TOX9552336	Rabbit NZW	6	NH ₄ -salt 90.8	0.5 g moistened with saline	Non irritant

NZW = New Zealand White

Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.

For details regarding studies reviewed during the 2001 EU evaluation please refer to the Monograph.

Reference: IIA, 5.2.4/01

Report: [REDACTED] 2007, Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)

[REDACTED] Study No.: B02294

Data owner: Nufarm

Date: 2007-03-01, unpublished

ASB2012-11418

Guidelines: OECD 404 (2002); Commission Directive 2004/73/EC B.4 (2004), JMAFF guideline 2-1-4 (2005)

Deviations: Yes

The test patch used had a surface of 16 cm² instead of 6 cm².

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate technical

Identification: NUP 05068

Description: Solid

Lot/Batch #: 200609062
Purity: 95.1%
Stability of test compound: Stable under storage conditions (20 ± 5 °C);
Expiry date: 2008-09-14
Vehicle and/
or positive control: Purified water
Test animals:
Species: Rabbit
Strain: New Zealand White, SPF
Source: [REDACTED]
Age: 13 weeks (male); 14 weeks (females)
Sex: One male and two females
Weight at dosing: 2.662 kg (male), 2.637 kg and 2.97 kg (females)
Acclimation period: At least five days
Diet/Food: Pelleted standard Provimi Kliba 3418 rabbit maintenance
diet (Provimi Kliba AG, CH-Kaiseraugust), *ad libitum*
Water: Tap water, *ad libitum*
Housing: Individually in stainless steel cages with feed hoppers and
drinking water bowls. Wood blocks and haysticks were
provided for gnawing.
Environmental conditions: Temperature: 17 - 23 °C
Humidity: 30 - 70 %
Air changes: 10 - 15/hour
12 hours light/dark cycle

Study design and methods

In life dates: 2007-01-04 to 2007-01-15

Animal assignment and treatment:

The test was conducted using young adult New Zealand albino rabbits (1 male, 2 females). The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance was moistened with approximately 0.5 mL of purified water was applied to the intact skin of the clipped left flank of the rabbits on an approx. 16-cm² gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed and the skin was cleaned with lukewarm tap water.

Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours after removal of the patch. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.

Results and discussion

Mortality: No mortality occurred.

Clinical observations: No clinical signs of systemic toxicity were observed during the study.

Body weight: All rabbits showed the expected body weight gain.

Necropsy: No necropsy was performed.

Skin observations: No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.

Conclusion by the Notifiers:

Based on the EU classification criteria, glyphosate technical (NUP 05068) is not to be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (NUP 05068) is also not classified for skin irritation.

Comment by RMS:

The study is considered acceptable and the tested substance not irritant.

Reference:

IIA, 5.2.4/02

Report:

2009, Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC In Rabbits

Data owner: Helm AG

Report No.: 24877

Date: 2009-11-27, unpublished

ASB2012-11419

Guidelines:

OECD 404 (2002); Commission Directive 2004/73/EC B.4 (2004), OPPTS 870.2500 (1998)

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Identification:

Glyphosate TC

Description:

White powder

Lot/Batch #:

2009051501

Purity:

96.4 %

Stability of test compound:

At room temperature in the dark stable until May 15, 2011.

Vehicle and/or positive control:

Purified water

Test animals:

Species:

Rabbit

Strain:

Himalayan

Source:

Age:

Approx. 7.5 - 8.5 months

Sex: Males
 Weight at dosing: 2.6 - 3.2 kg
 Acclimation period: At least 20 days.
 Diet/Food: ssniff K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period
 Water: Tap water, *ad libitum* before and after the exposure period
 Housing: Individual housing
 Environmental conditions: Temperature: 20 ± 3 °C
 Humidity: 30 - 70 %
 Air changes: no data
 12 hours light/dark cycle

Study design and methods

In life dates: 2009-10-15 to 2009-10-23

Animal assignment and treatment:

The test was conducted using three young male adult Himalayan albino rabbits. The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance was moistened with purified water and applied to the intact skin of the rabbits on an approx. 6-cm² gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed. No residual test item had to be removed.

Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours after removal of the patch.

Results and discussion

Mortality: No mortality occurred.

Clinical observations: No clinical signs of systemic toxicity were observed during the study.

Body weight: All rabbits showed the expected body weight gain.

Necropsy: No necropsy was performed.

Skin observations: No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.

Conclusion by the Notifiers

Based on the scores for erythema and oedema and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, glyphosate TC is not to be classified for skin irritation.

Comment by RMS:

The study is considered acceptable and the tested substance not irritant.

Reference:

IIA, 5.2.4/03

Report:

1995, HR-001: Primary Dermal irritation study in rabbits.

Report No.: IET 95-0035

Guidelines: Data owner: Arysta LifeScience
Date: 1995-06-28, unpublished
ASB2012-11420
U.S. EPA FIFRA Guideline Subdivision F
MAFF 59 NohSan No. 4200 (1985)
Draize method

Deviations: None

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material: Glyphosate technical

Identification: HR-001

Description: White crystal

Lot/Batch #: T-941209

Purity: 97.56 %

Stability of test compound: Not mentioned in the report

Vehicle and/or positive control: Deionised water

Test animals:

Species: Rabbit

Strain: New Zealand White, SPF

Source: [REDACTED]

Age: 12 weeks

Sex: Six females

Weight at dosing: 2.512 (2408 – 2686) kg

Acclimation period: 18 days

Diet/Food: Pellet Diet GC4 (Oriental Yeast Co., Ltd.)

Water: Water filtrated and sterilized, *ad libitum*

Housing: Individually in stainless steel cages with feed hoppers and drinking water bowls.

Environmental conditions: Temperature: 23.9 - 24 °C
Humidity: 52.8 – 56.6 %
Air changes: 15/hour
12 hours light/dark cycle

Study design and methods

In life dates: 1995-05-16 – 1995-05-19

Animal assignment and treatment:

Glyphosate (0.5 g) moistened with 0.5 mL of deionised water was then applied to the closely-clipped dorso-lumbar region of 6 New Zealand rabbits and covered by a semi-occlusive gauze patch for 4 hours. At the end of the exposure period, the patch was removed and the treatment site was washed with distilled water to remove any residual test substance. All animals were observed for primary dermal irritation 1, 24, 48 and 72 hours after removal of the patch. Degree of erythema and edema relative to treatment were recorded during a subsequent 72-

hour observation period. Body weights were measured prior to application, and after the final observation.

Results and discussion

Mortality: No mortality occurred.

Clinical observations: Clinical signs were not observed.

Body weight: All rabbits showed the expected body weight gain.

Necropsy: No necropsy was performed.

Skin observations: : No signs of erythema, eschar, edema and any other evidence of irritation were observed in either the test substance treated site or the negative control site at any time during the observation period.

The observation period was therefore completed after 72 hours.

Conclusion by the Notifiers

Based on the EU classification criteria, glyphosate technical (HR-001) is not to be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (HR-001) is also not classified for skin irritation.

Comment by RMS:

The study is considered acceptable and the tested substance not irritant.

Reference:

IIA, 5.2.4/04

Report:

2009, Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits

Data owner: Helm AG

Report No.: LPT 23913

Date: 2009-04-30, unpublished

ASB2012-11421

Guidelines:

OECD 404 (2002)

US EPA OPPTS 870.2500

Deviations:

Personnel change of the head of the Quality Assurance Unit:
until January 31, 2009: Dipl. Biol. S. Steuer
as of February 1, 2009: Dr. med. vet. K. R. habil. Sultan. This minor deviation did not have any effect on the validity and integrity of the scientific results obtained in this study

GLP:

yes

Acceptability:

Please see comment by RMS.

Materials and methods

Test material:

Glyphosate

Identification:

Glyphosate TC

Description:

White powder

Lot/Batch #:

20080801

Purity:

98.8 %

Stability of test compound: No data given in the report.

Vehicle and/or positive control: Deionised water

Test animals:

Species: Rabbit

Strain: Himalayan

Source: [REDACTED]

Age: 24601 Löhndorf/Post Wankendorf, Germany

Sex: Approx. 4 - 5 months

Weight at dosing: Male animals

Animal no. 1: 4.4 kg

Animal no. 2: 4.0 kg

Animal no. 3: 3.8 kg

Acclimation period: At least 20 adaptation days

Diet/Food: Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available *ad libitum* before and after the exposure period.

Water: Tap water, *ad libitum*

Housing: The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany).

Environmental conditions: Temperature: 20 ± 3 °C

Humidity: 30 - 70 %

12-hour light/dark cycle

Study design and methods

In life dates: 2009-02-04 to 2009-02-13

Animal assignment and treatment:

Approximately 24 hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.

A dose of 500 mg of the test item was applied to the test site (area: approx. 6 cm²).

The test item was applied to the test site and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control.

Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers.

At the end of the exposure time no residual test item had to be removed. As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours.

As neither a corrosive effect nor a severe irritant effect was observed after a four hour exposure, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72 hours after patch removal. None of the three rabbits showed any significant test item-related lesions at these examination time points.

Conclusion by the Notifiers

Under the present test conditions, none of three rabbits exposed for 4 hours to 500 mg Glyphosate TC/patch (semi-occlusive conditions) showed any test item-related changes. There were no systemic intolerance reactions.

According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions, Glyphosate TC was non - irritating to skin, hence, no labelling is required.

Comment by RMS:

The study is considered acceptable and the tested substance not irritant.

Reference:

IIA, 5.2.4/05

Report:

2010, Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits

Data owner: Helm AG
Report No.: LPT 24605
Date: 2010-01-06, unpublished
ASB2012-11422

Guidelines:

OECD 404 (2002)
US EPA OPPTS 870.2500

Deviations:

No deviations from the Study Plan

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Deionised water
Test animals:	
Species:	Rabbit
Strain:	Himalayan

Source:



Age: Approx. 6 - 7 months

Sex: Male animals

Weight at dosing: Animal no. 1 : 2.9 kg

Animal no. 2: 2.4 kg

Animal no. 3: 2.5 kg

Acclimation period: At least 20 adaptation days

Diet/Food: Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available ad libitum before and after the exposure period.

Water: Tap water, *ad libitum*

Housing: The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany).

Environmental conditions: Temperature: 20 ± 3 °C

Humidity: 30 - 70 %

12-hour light/dark cycle

Study design and methods:

In life dates: 2009-10-26 to 2009-11-06

Animal assignment and treatment:

Approximately 24 hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.

A dose of 500 mg of the test item was applied to the test site (area: approx. 6 cm²).

The test item was applied to the test site and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control.

Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers.

At the end of the exposure time no residual test item had to be removed. As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours.

As neither a corrosive effect nor a severe irritant effect was observed after a four hour exposure, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72 hours after patch removal. None of the three rabbits showed any significant test item-related lesions at these examination time points.

Conclusion by the Notifiers

Under the present test conditions, none of three rabbits exposed for 4 hours to 500 mg Glyphosate TC/patch (semi-occlusive conditions) showed any test item-related changes. There were no systemic intolerance reactions.

According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions, Glyphosate TC was non - irritating to skin, hence, no labelling is required.

Comment by RMS:

The study is considered acceptable and the tested substance not irritant.

Reference: IIA, 5.2.4/06
Report: [REDACTED] 2009, Glyphosate – Acute Dermal Irritation Study in Rabbits
 [REDACTED]
 Report No.: 12173-08
 Date: 2009-03-11, unpublished
 ASB2012-11423
Guidelines: US EPA OPPTS 870.2500
 Equivalent to OECD 404 (2002).
Deviations: Humidity was in the range of 43-92% instead of 30-70%. This deviation did not affect the study outcome
GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate
 Identification: Glyphosate Tech Grade Mixed 5-Batch
 Description: White powder
 Lot/Batch #: 080704-1 thru 5
 Purity: 96.4%
 Stability of test compound: No data given in the report.
 Vehicle and/or positive control: Deionised water
 Test animals:
 Species: Albino rabbit
 Strain: New Zealand White
 Source: [REDACTED]
 Age: Approx. 3 months
 Sex: 1 male and 2 females (nulliparous and non-pregnant)
 Weight at dosing: Male: 2.000 kg; Females: 2.600 kg
 Acclimation period: 5 days
 Diet/Food: PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily

Water: Tap water, *ad libitum*
 Housing: Individual housing in suspended, wire bottom, stainless steel cages.
 Environmental conditions: Temperature: 20 ± 3 °C
 Humidity: 30 - 70 %
 Air changes: 10 - 12/hour
 12-hour light/dark cycle

Study design and methods:

In life dates: 2008-11-03 to 2008-11-14

Animal assignment and treatment:

Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 x 8 cm. Only those animals with exposure areas free of pre-existing skin irritation or defects were selected for testing. A single intact exposure site was selected as the test site while the contralateral intact site served as a control site.

On Day 0, 500 mg of test substance moistened with 0.2 mL of deionized water was applied to each test site and covered with a 4 ply, 2.5 x 2.5 cm surgical gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopedic stockinette) which was secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance. After four hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The test sites were observed for erythema and edema formation, and any other dermal defects or irritation at 1, 24, 48 and 72 hours after unwrap.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Conclusion by the Notifiers

The primary irritation index of 0.0 out of a possible 8.0 was obtained from the 1, 24, 48 and 72 hour observations and was used to give Glyphosate a descriptive rating of non-irritating. Based on the 72-hour observations only, Glyphosate is assigned to Toxicity Category IV.

Comment by RMS:

The study is considered acceptable and the tested substance not irritant.

Reference:

IIA, 5.2.4/07

Report:

2005, Glyphosate Acid Technical – Primary Skin Irritation Study in Rabbits

Report No.: PSL 15278

Date: 2005-04-04, unpublished

ASB2012-11424

Guidelines:

US EPA OPPTS 870.2500

Equivalent to OECD 404 (2002).

Deviations:

None

GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate
 Identification: Glyphosate Acid Technical
 Description: White crystalline powder
 Lot/Batch #: 040205
 Purity: 97.23 %
 Stability of test compound: No data given in the report.
 Vehicle and/or positive control: Distilled water
 Test animals:
 Species: Rabbit
 Strain: New Zealand albino
 Source: [REDACTED]
 Age: Young adult
 Sex: Male
 Weight at dosing: No data given in the report.
 Acclimation period: 21 days
 Diet/Food: Pelleted Purina Rabbit Chow #5326
 Water: Tap water, *ad libitum*
 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week
 Environmental conditions: Temperature: 18-22 °C
 12-hour light/dark cycle

Study design and methods

In life dates: 2004-05-05 to 2004-05-08

Animal assignment and treatment:

On the day before application, a group of animals was prepared by clipping (Oster model #A5-small) the dorsal area and the trunk. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy animals without preexisting skin irritation were selected for test. Prior to application, the test substance was moistened with distilled water to achieve a dry paste by preparing a 70 % w/w mixture. Five-tenths of a gram of the test substance (0.71 g of the test mixture) was placed on a 1-inch x 1-inch, 4-ply gauze pad and applied to one 6-cm² intact dose site on each animal. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3-inch Micropore tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.

After 4 hours of exposure to the test substance, the pads and collars were removed and the test sites were gently cleansed of any residual test substance. Individual dose sites were scored according to the Draize scoring system at approximately 1, 24, 48, and 72 hours after patch removal.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: All animals appeared active and healthy. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. Two of three sites were free from irritation throughout the study. One hour after patch removal, one animal exhibited very slight erythema. Dermal irritation cleared from this animal by 24 hours.

Conclusion by the Notifiers

Under the conditions of this study, Glyphosate Acid Technical is classified as slightly irritating to the skin.

Comment by RMS:

The study is considered acceptable. The test substance provoke very slight erythema in one animal one hour after patch removal, which was cleared within 24 hours. According to current EU and GHS criteria classification as 'irritating to skin' is not warranted.

Reference: IIA, 5.2.4/08

Report: [REDACTED] 2008, Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical [REDACTED]

Report No.: RF-3996.311.476.07

Date: 2008-09-23, unpublished

ASB2012-11425

Guidelines: OECD 404 (2002).

Deviations: The experimental phase initiation and experimental phase conclusion dates were updated

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate

Identification: Glyphosate Technical

Description: Solid

Lot/Batch #: 20070606

Purity: 98.05 %

Stability of test compound: No data given in the report.

Vehicle and/or positive control: Deionised water

Test animals:

Species: Rabbit

Strain:	New Zealand White
Source:	
Age:	17 weeks
Sex:	Female
Weight at dosing:	2.907 - 3.145 kg
Acclimation period:	5 to 6 days
Diet/Food:	Pelleted and autoclaved commercial diet for rabbits (<i>Guabi, Mogiana Alimentos S.A. - Brazil</i>)
Water:	Tap water, <i>ad libitum</i>
Housing:	The animals were housed individually in galvanized steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.
Environmental conditions:	Temperature: 17 - 22 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods

In life dates: 2008-05-20 to 2008-05-24

Animal assignment and treatment:

Each animal provisionally selected for the test was prepared by clipping the fur from the back approximately 24-hr prior to the application of the test item, using a small animal clipper (Oster model Golden A5, Electric Razor) with great care taken to avoid abrading the skin during the clipping procedure, so as not to alter its permeability. The clipped area was large enough to allow clear visualisation of the test site. After being clipped, visual examination of the skin confirmed the skin was intact and healthy. 0.5 g of the test item was applied over the skin of each animal. The test item was first placed onto a moistened gauze dressing, which was applied over a small section of the test area (approximately 6 cm²) in such a manner that there was good contact and uniform distribution of the test item on the skin. After application, the gauze was held in the test site by an adhesive and non-irritating tape. Removal and ingestion of the test item was prevented by placing a suitable adhesive tape (semi-occlusive dressing) around the trunk and test area. Adjacent untreated shaved areas of the skin were used as the negative control. After the 4-hour exposure period, the gauze patches were removed, any residual test item washed using physiological saline and then the treated areas examined for signs of irritation at specified intervals. The test was performed initially using one single animal for evaluation of any irritant/corrosive effect of the test item to the skin. Because no severe dermal reaction was observed in the initial test, two additional animals were tested to confirm the response.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Animals' skin was examined for signs of erythema, eschars and edema formation at approximately 1, 24, 48 and 72 hours after removal of the patch. No signs of dermal irritation were observed at any of the time points in any of the animals tested.

Body weight: All animals presented gain in body weight during the observation period.

Conclusion by the Notifiers:

Under the test conditions, the test item Glyphosate technical when applied on the skin of the rabbits did not cause any dermal irritation. No treatment-related behavioral or clinical alterations were noted during the observation period.

Comment by RMS:

The study is considered acceptable and the test substance not irritant.

Reference:

IIA, 5.2.4/09

Report:

1988b, Primary Dermal Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits

Data owner: Monsanto
Monsanto Report No.: FD-88-29
Date: 1988-06-08, Unpublished
Z35394

Guidelines:

US EPA 81-5

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Identification:

Glyphosate

Description:

White powder

Lot/Batch #:

XLI-55

Purity:

97.76 %

Stability of test compound:

Stored at room temperature

Vehicle and/or positive control:

Physiological saline

Test animals:

Species:

Rabbit

Strain:

New Zealand White

Source:

Age:

Young adult

Sex:

Three males and three females

Weight at dosing:

Between 2 and 3 kg

Acclimation period:

At least five days

Diet/Food:

NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*

Water:

Tap water, *ad libitum*

Housing:

Individually in stainless steel mesh cages

Environmental conditions: Temperature: 20 – 23.9 °C
 Humidity: 40 - 60 %
 Air changes: Not specified
 Light cycle: 12 hours light/dark cycle

Study design and methods:

In life dates: 1998-04-11 to 1998-04-14

Animal assignment and treatment:

Six healthy animals weighing between two and three kilograms were selected randomly from the acclimated colony and assigned to the test group. Selection suitability was based on health, weight requirement and of dorsal skin for testing. The fur on the back of each rabbit was clipped with an electric clipper on the day prior to dose administration. The test article (0.5 g moistened with 0.5 mL physiological saline) was applied topically to each of two intact dorsal test sites per rabbit. Immediately after dosing, the test sites were semi-occluded with a one-inch square gauze patch held in place with tape. The animals were collared during the exposure period to prevent removal of the patches. The patches and collars were removed four hours after dose administration and the exposure sites gently wiped with gauze to remove as much non-absorbed test article as possible.

Dermal irritation was evaluated at 0.5, 24, 48 and 72 hours after patch removal. Erythema and edema were scored separately according to the Draize method. The animals were observed twice daily for mortality at least five hours apart. Body weights were obtained on study day 1 prior to dose administration. At study termination, the animals were euthanised by intra-cardiac injection of sodium pentobarbital and discarded.

A mean primary irritation score was calculated at each scoring interval from individual scores obtained from the test animals. Six individual animal scores were calculated from dermal irritation readings taken at 0.5, 24, 48 and 72 hours after patch removal. Individual animal scores were obtained at each scoring interval by adding the total erythema and eschar formation scores from both application sites to the total edema formation scores from both sites and dividing by two. The mean of the six individual animal scores represents the mean primary irritation score at each interval.

Results and discussion

Mortality: No mortality occurred.

Clinical observations: Not reported.

Body weight: Not reported.

Necropsy: No necropsy was performed.

Skin observations: No dermal irritation was noted following test substance application.

Conclusion by the Notifiers:

Based on the EU classification criteria, glyphosate is not to be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate is also not classified for skin irritation.

Comment by RMS:

The study is considered acceptable and the test substance not irritant.

Reference: IIA, 5.2.4/10

Report: [REDACTED] 1979, Primary

Dermal Irritation in Rabbits

[REDACTED]

Monsanto Report No.: BND-77-428

Date: 1979-08-06, Unpublished

Z35544

Guidelines: Not specified
Deviations: Not specified
GLP: no (pre-GLP)
Acceptability: *Please see comment by RMS*

Materials and methods

Test material:

Identification: Glyphosate Technical

Description: Fine white powder

Lot/Batch #: XLI-180

Purity: 99 %

Stability of test compound: Not specified

Vehicle and/or positive control: Distilled water

Test animals:

Species: Rabbit

Strain: New Zealand White

Source: [REDACTED]

Age: Not specified

Sex: Three males and three females

Weight at dosing: 2.25 – 2.80 kg

Acclimation period: Not specified

Diet/Food: Not specified

Water: Not specified

Housing: Not specified

Environmental conditions: Temperature: Not specified
Humidity: Not specified
Air changes: Not specified
Light cycle: Not specified

Study design and methods:

In life dates: Not specified.

Animal assignment and treatment:

Six albino rabbits were closely clipped over the back and sides with an electric clipper. There were four test sites per rabbit, each site 1" x 1" in area. Two sites, one on each side of the spinal column were abraded, while the remaining two sites were left intact. The abrasions were sufficiently deep so as to penetrate the stratum corneum, but not so deep as to disturb the derma or produce bleeding.

The test material was administered as a 25 % w/v solution in distilled water. In all cases 0.5 mL of the test substance was applied beneath a surgical gauze square, 1" x 1", eight single layers thick, placed directly on the test site and secured with tape. The animals were then wrapped with plastic sheeting secured with masking tape to help contain the test material. After 24 hours the sheeting and gauze patches were removed.

Observations for signs of dermal irritation or systemic toxicity were recorded at 24 and 72 hours after application. At each observation all treated sites were scored for erythema, edema and eschar formation

Results and discussion

Mortality: No mortality occurred.

Clinical observations: Not reported.

Body weight: Not reported.

Necropsy: No necropsy was performed.

Skin observations: In the intact skin, one animal had very slight erythema (score of 1) at 24 hours following test substance application. There was no other irritation noted for intact skin at 24 or 72 hours.

Conclusion by the Notifiers

Based on the EU classification criteria, glyphosate should not be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate is also not classified for skin irritation.

Comment by RMS:

The study is considered not acceptable due to several deficiencies (pre-GLP, no guideline, observation period too short, clinical signs & body weight not reported).

Reference: IIA, 5.2.4/11

Report: [REDACTED] 1996, Glyphosate Acid: Skin Irritation to the Rabbit

Data owner: Syngenta

Report No.: [REDACTED]/P/4695

Date: 1996-08-23, not published

TOX2000-1985

Guidelines: OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6 % w/wi
CAS#:	Not reported
Stability of test compound:	The test substance was used within the expiry date
Vehicle and/or positive control:	Deionised water (for moistening)
Test Animals:	
Species	Rabbit
Strain	New Zealand White albino
Age/weight at dosing	Young adult / 3001-4386 g
Source	[REDACTED]
Housing	Individually in aluminium sheet cages in racks suitable for animals of this strain and the weight range expected during the course of the study.
Acclimatisation period	At least 6 days
Diet	STANRAB SQC, (Special Diet Services Limited, Stepfield, Witham, Essex, UK) <i>ad libitum</i>
Water	Mains water <i>ad libitum</i>
Environmental conditions	Temperature: 17±2 °C Humidity: 40-70 % Air changes: Approximately 25/hour Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 9 March 1995 End: 28 April 1995

Animal assignment and treatment: In a primary skin irritation study, Glyphosate acid (95.6 % w/w) was applied by semi-occlusive application of 500 mg to the intact skin of the left flank of each of six female, young adult New Zealand White rabbits.

Approximately one day before treatment, the left flank was clipped with an electric clipper, exposing an area of approximately 7 cm x 13 cm.

On the day of treatment, 500 mg of glyphosate acid (95.6 % w/w) (moistened with approximately 0.5 mL deionised water) was applied to the test site (approximately 2.5 cm x 2.5 cm) on the left flank of the rabbit. The treated area was covered with a piece of 8-ply surgical gauze (approximate size 2.5cm x 2.5cm), which was secured by two strips of surgical tape (approximate size 1 cm x 8cm). This was covered by a piece of impermeable rubber sheeting (approximate size 35cm x 13cm) wrapped once around the trunk of the animal and secured with adhesive impermeable polyethylene tape (7.5cm wide).

The dressings were left in position for approximately four hours. The application site was gently cleansed free of any residual test substance using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

The Draize scale was used to assess the degree of erythema and oedema at the application sites approximately 30-60 minutes, 1, 2 and 3 days after removal of the dressings. Any other signs of skin irritation were also noted.

Results and discussion

There were no signs of ill-health and no signs of skin irritation in any animal during the study.

Table B.6.2-22: Individual and mean skin irritation scores of glyphosate acid according to the Draize scheme

Time	Erythema						Oedema					
Animal number	1	2	3	4	5	9	1	2	3	4	5	9
after 1 hour	0	0	0	0	0	0	0	0	0	0	0	0
after 24 hours	0	0	0	0	0	0	0	0	0	0	0	0
after 48 hours	0	0	0	0	0	0	0	0	0	0	0	0
after 72 hours	0	0	0	0	0	0	0	0	0	0	0	0
mean score 24-72 h	0	0	0	0	0	0	0	0	0	0	0	0

Conclusion by the Notifiers:

Glyphosate acid is non-irritant following a single four-hour application to rabbit skin.

Comment by RMS:

The study is considered acceptable and the test substance is considered not irritant.

Reference:

IIA, 5.2.4/12

Report:

2007, Glyphosate Technical Material: Primary Skin Irritation Study In Rabbits (4-Hour Semi-Occlusive Application)

Data owner: Syngenta

Report No.: R61837/1010

Date: 2007-02-08, not published

ASB2012-11426

Guidelines:

OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test Material:

Glyphosate Technical Material

Description:

Technical, white powder

Lot/Batch number:

0507

Purity:

96.1 % w/w glyphosate acid

CAS#:

Stability of test compound:

Stable under storage conditions of room temperature (range of 20 °C ± 5 °C), protected from light and humidity.

Vehicle and/or positive control:

The test substance was moistened with purified water before application.

Test Animals:

Species

Rabbit

Strain	New Zealand White (SPF)
Age/weight at dosing	Male: 10-11 weeks / 2440 g; Females: 15-16 weeks / 2749 and 2815 g
Source	[REDACTED]
Housing	Individually in stainless steel cages equipped with feed hoppers and drinking water bowls.
Acclimatisation period	5/6 days
Diet	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet <i>ad libitum</i> .
Water	Community tap water <i>ad libitum</i>
Environmental conditions	Temperature: 17-23 °C Humidity: 30-70 % Air changes: 10-15 per hour. Photoperiod: 12 hours light, 12 hours dark.

Study design and methods

In-life dates: Start: 18 December 2006 End: 22 December 2006

Animal assignment and treatment: Three young adult (1 male and two female) New Zealand White rabbits were used in the study. As it was suspected that the test substance might produce irritancy, a single animal (one female) was treated first. As no corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals for an exposure period of 4 hours.

Four days before treatment, the left flank was clipped, exposing an area of approximately 100 cm² (10 cm x 10 cm). The skin of the animals was examined one day before treatment, and regrown fur of all animals was clipped again. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5 g of Glyphosate Technical Material was placed on a surgical gauze patch (2.5 cm x 2.5 cm). This gauze patch was applied to the intact skin of the clipped area. The patch was covered with a semi-occlusive dressing which was wrapped around the abdomen and anchored with tape.

The duration of treatment was 4 hours after which the dressing was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible at that time.

Observations for viability, mortality and clinical signs were carried out daily from acclimatisation of the animals to the termination of the study. Bodyweights of individual animals were recorded at the start of the acclimatisation period, on the day of application and at termination of the observation period.

The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, approximately 1, 24, 48 and 72 hours, after removal of the dressing, gauze patch and test substance. To evaluate the irritation potential of the test substance, the mean values of erythema/eschar and oedema formation were calculated for each animal using the scores between 24 and 72 hours. The Primary Irritation Index (P.I.I.) was calculated by adding together the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of available figures.

Results and discussion

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. The test substance did not elicit any skin reactions at the application site of any animal at any of the observation times (all scores 0). The individual mean score for erythema/eschar and oedema for each of the three animals was therefore 0. No staining produced by the test item of the treated skin was observed and no alterations of the treated skin or corrosive effects were evident. The bodyweights of the rabbits were considered to be within the normal range of variability.

Conclusion by the Notifiers:

The application of Glyphosate Technical Material to the intact skin resulted in no signs of irritation. According to Draize classification criteria Glyphosate Technical Material is considered to be “not irritant” to rabbit skin (P.I.I. = 0.00).

Comment by RMS:

Under the present conditions the study is considered acceptable and the tested substance not irritant.

Reference: IIA, 5.2.4/13

Report: [REDACTED] 2011, Glyphosate technical: Primary skin irritation study in rabbits

Data owner: Syngenta

Report No.: 10/218-006N

Date: 2011-04-13

not published

ASB2012-11427

Guidelines: OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test Material: Glyphosate technical

Description: Technical, dry white powder

Lot/Batch number: 569753 (BX20070911)

Purity: 96.3 % w/w Glyphosate technical

CAS#: Not reported

Stability of test compound: Stable under storage conditions (room temperature range <30 °C), recertification date end August 2011

Vehicle and/or positive control: None

Test Animals:

Species: Rabbit

Strain: New Zealand White

Age/weight at dosing: Approximately 12 weeks / 2995-3095 g

Source: [REDACTED]

Housing: Individually in metal cages

Acclimatisation period	5 days
Diet	Purina Base – Lap gr. diet (AgribandsEurope Hungary PLC, H-5300 Karcag, Madarasi út, Hungary) <i>ad libitum</i>
Water	Municipal tap water <i>ad libitum</i>
Environmental conditions	Temperature: 17-20 °C Humidity: 30-70 % Air changes: 15-20/hour Photoperiod: 12 hours light/12 hours dark

Study design and methods

In-life dates: Start: 02 November 2010 End: 05 November 2010

Animal assignment and treatment: In a primary dermal irritation study, three male, young adult, New Zealand White rabbits were each given a dermal application of 0.5 g of undiluted Glyphosate Technical (96.3 % w/w Glyphosate technical).

Approximately 24 hours prior to the test the hair was clipped from the back and flanks of the animals with an electric clipper, exposing an area approximately 10 cm x 10cm. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5g of glyphosate technical was placed on a surgical gauze pad (approximately 2.5 cm x 2.5 cm). This gauze pad was applied to the intact skin of the clipped area and was kept in contact with the skin by a patch with a surrounding adhesive hypoallergenic plaster. The entire trunk of the animals was then wrapped with plastic wrap held in place with an elastic stocking.

The dressing was left in place for 4 hours after which it was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible.

As it was suspected that the test item might produce irritancy, a single animal was treated first. As no corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals.

The animals were checked daily for signs of systemic toxicity and mortality. Body weights were recorded on the day of application and at termination of observations.

The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004, which was based on the Draize scoring system approximately 1, 24, 48 and 72 hours after the removal of the dressing, gauze patch and test item. The mean score was calculated across 3 scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately. An animal was positive when the mean score was 2 or greater. The test was positive for irritation when at least 2 animals were positive for the same endpoint (erythema/eschar or oedema).

The Cumulative Scores for the Skin Irritation Scores were calculated and represent the sum of all numerical scores for each animal at each time point. The resulting Mean Cumulative Skin Irritation Score was calculated for all animals at each time point.

The Primary Irritation Index (P.I.I.) was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of available figures.

Results and discussion

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. The bodyweights of all rabbits were considered to be within the normal range of variability. There was no staining of the skin, alterations or corrosive effects.

At the observations 1 and 24 hours after patch removal, very slight erythema (score 1) was observed in one animal. No signs of irritation were observed in the other treated animals throughout the study.

As no signs of irritation were observed 72 hours after patch removal, the study was terminated after the 72 hour observation.

Table B.6.2-23: Individual and mean skin irritation scores of glyphosate technical

Time	Erythema			Oedema		
	606	622	620	606	622	620
Animal number	606	622	620	606	622	620
after 1 hour	0	1	0	0	0	0
after 24 hours	0	1	0	0	0	0
after 48 hours	0	0	0	0	0	0
after 72 hours	0	0	0	0	0	0
mean score 24-72 h	0	0.3	0	0	0	0

Conclusion by the Notifiers:

According to the Draize classification criteria, glyphosate technical is considered to be a “mild-irritant” to rabbit skin (P.I.I. = 0.11).

Comment by RMS:

The study is considered acceptable. The test substance provoke slight erythema in one animal one hour after patch removal, which was cleared within 48 hours. According to current EU and GHS criteria classification as ‘irritating to skin’ is not warranted.

Comment by GTF on the first draft of the RAR (July 2013):

RMS stated: “Three of the new studies revealed mild or slight irritating effects.

GTF: non-irritant to intact skin and only slightly irritant to abraded skin.

RMS comment (August 2013):

It is clearly stated that no classification and labelling is necessary and that in the majority of studies no evidence of skin irritation was obtained. Furthermore, studies conducted by [REDACTED] (2005, ASB2012-11424) and [REDACTED] (2011, ASB2012-11427) showed slight or mild effects at intact skin.

B.6.2.5 Eye irritation

Eye irritation was examined in numerous studies in rabbits that were performed either with the acid or its different salts. In addition to the previous evaluation a huge number of further studies were provided for the current re-evaluation. To conclude on all study results, glyphosate acid was tested to be a strong eye irritant and classification and labelling (Xi/R41 or Cat. 1, H318) is needed.

Table B.6.2-24: Summary of eye irritation studies with glyphosate acid

Reference (Data owner)		Species Strain	Number and /or sex of animals	Purity [%]	Amount applied Exposure conditions	Effects / Result
Studies from the 2001 evaluation	Annex B-5.2.6.1, Glyphosate Monograph [REDACTED] 1994 (Herbex) TOX9500249	Rabbit NZW	1 ♀	95	0.1 mL (ca. 76 mg)	Due to strong ocular effects the test was stopped for humane reasons after 1 hour irritant
	Annex B-5.2.6.1, Glyphosate Monograph [REDACTED] 1994 (Alkaloida) TOX9650146	Rabbit NZW	4 ♀	97.2	100 mg (pure)	Significant ocular lesions, especially chemosis irritant
	Annex B-5.2.6.1, Glyphosate Monograph [REDACTED], 1991 (FSG) TOX9551093	Rabbit NZW	2 ♂, 1 ♀	96.8	100 mg	Mortality (1 ♀: severe enteritis and opacity); significant ocular lesions that were not reversible within 3 weeks moderately irritating
	Annex B-5.2.6.1, Glyphosate Monograph [REDACTED], 1991 (I. Pi. Ci.) Z101610	Rabbit NZW	3 ♂	98	100 mg (pure)	Several ocular effects (score 1 & 2) that were reversible within 3 days slightly irritant
Studies from the 2001 evaluation	Annex B-5.2.6.1, Glyphosate Monograph [REDACTED], 1990 (AGC) TOX9500264	Rabbit NZW	3 ♀	98.1	100 mg	Corneal opacity not reversible within 7 days (2 ♀), iris lesions not reversible within 6 days (1 ♀), conjunctival redness not reversible within 6 days (3 ♀) slightly irritant
	Annex B-5.2.6.1, Glyphosate Monograph [REDACTED], 1989 (CHE) TOX9552338	Rabbit NZW	1 ♂	98.6	100 mg (pure)	Due to strong ocular effects the test was stopped for humane reasons after treatment of the first of six animals moderately to severely irritating
	IIA 52.5 [REDACTED] 1996 (CHE) TOX1999-881	Rabbit NZW	6 ♂, 3 ♀	98.2	65 mg one group (3 ♂, 3 ♀) eyes were not washed; one group (3 ♂) eyes were washed 30 sec after treatment	Severely irritating (washed eyes) moderately irritating (non-washed eyes)

Reference (Data owner)		Species Strain	Number and /or sex of animals	Purit y [%]	Amount applied Exposure conditions	Effects / Result
Studies not reviewed in the 2001 evaluation	IIA 5.2.5/01 [REDACTED] 2007 (NUF) ASB2012- 11428	Rabbit NZW	1 ♂ 2 ♀	95.1	100 mg	Marked, early-onset and transient ocular changes, like very slight to slight corneal opacity, slight to marked conjunctival redness, conjunctival chemosis, reddening of the sclera and discharge. All eye effects were reversible within 10 days after instillation. No signs of corrosion or staining were observed in any eye.
	IIA 5.2.5/02 [REDACTED], 2009 (HAG) ASB2012- 11429	Rabbit Himalaya n	3 ♂	96.4	100 mg (eyes were rinsed 1 h post- application) (study considered supplementary)	slight and fully reversible ocular changes (\leq grade 1); all eye effects were reversible within 8 days after instillation. No signs of corrosion or staining were observed in any eye Non irritant
	IIA 5.2.5/03 [REDACTED] 1995 (ALS) ASB2012- 11430	Rabbit NZW	12 ♀	97.56	100 mg (pure) (one group without irrigation of eyes, two groups with irrigation of eyes at different time intervals after application)	Severely irritating; animals in the irrigation groups showed reduced eye irritation and faster recovery
	IIA 5.2.5/04 [REDACTED] 2009 (EXC) (Expert statement) ASB2012- 11431	Rabbit	Not applicable	96.66	Test solution: 1% in purified water	Not performed since pH of test item solution was < 2 (corrosive)
Studies not reviewed in the 2001 evaluation	IIA 5.2.5/05 [REDACTED] 2009 (HAG) ASB2012- 11432	Rabbit Himalaya n	3 ♂	98.8	100 mg (eyes were rinsed 1 h post- application) (study considered supplementary)	Non irritant
	IIA 5.2.5/06 [REDACTED] 2010 (HAG) ASB2012- 11433	Rabbit Himalaya n	3 ♂	97.3	100 mg (eyes were rinsed 1 h post- application) (study considered supplementary)	Non irritant

Reference (Data owner)	Species Strain	Number and /or sex of animals	Purit y [%]	Amount applied Exposure conditions	Effects / Result
IIA 5.2.5/07 [REDACTED] 2009 (HAG) ASB2012- 11434	Rabbit NZW	2 ♂, 1 ♀	96.4	0.1 mL (93.2 mg)	Irritant
IIA 5.2.5/08 [REDACTED] 2005 (HAG) ASB2012- 11435	Rabbit NZW	3 ♂	97.23	0.1 mL (60 mg)	Irritant
IIA 5.2.5/09 [REDACTED], 2008 (HAG) ASB2012- 11436	Rabbit NZW	1 ♂ 2 ♀	98.05	100 mg	Severely irritating
IIA 5.2.5/10 [REDACTED] 1988 (MON) Z35395	Rabbit NZW		97.76		Produced corneal opacity and conjunctival irritation with blistering, irritation persisted in 4/5 rabbits on day 21
IIA 5.2.5/11 [REDACTED] 1997 (SYN) TOX2000-1986	Rabbit NZW	6 ♀	95.6	100 mg	Irritant
IIA 5.2.5/12 [REDACTED] 2007 (SYN) ASB2012- 11437	Rabbit NZW	1 ♂ 2 ♀	96.1	100 mg	Slight conjunctival redness, conjunctival chemosis, reddening of the sclera and discharge were observed. Effects reversible and no longer evident 7 days after treatment.
IIA 5.2.5/13 [REDACTED] 2011 (SYN) ASB2012- 11438	Rabbit NZW	2 ♂	96.3	100 mg	Corrosive. Due to strong ocular effects the test was stopped for humane reasons after 24 hours.

NZW = New Zealand White

Table B.6.2-25: Summary of eye irritation studies with glyphosate salts

Reference (Data owner)	Species Strain	Number and /or sex of animal TOXs /	Purity [%]	Amount applied / Exposure conditions	Results
Annex B- 5.2.6.2, Glyphosate Monograph [REDACTED] 1994 (MON / CHE) TOX9552340	Rabbit NZW	1 ♂, 5 ♀	IPA 62%	0.1 mL (pure) (pre-treatment with local anaesthetic)	Iris, conjunctivae: redness & chemosis within 1 h; discharge after 1 h Slightly irritating
Annex B- 5.2.6.2, Glyphosate Monograph [REDACTED] 1989 (I.Pi.Ci.) TOX9551629	Rabbit NZW	3 ♂, 3 ♀	IPA 62	0.1 mL; ♂: eyes unrinsed; ♀: eyes rinsed	Conjunctival redness (24 h) Slightly irritating
Annex B- 5.2.6.2, Glyphosate Monograph [REDACTED] 1987 (MON/CHE) TOX9552342	Rabbit NZW	6	NH ₄ - salt 90.8	0.1 g	conjunctivae: redness & chemosis (48h); discharge in all animals within 48 h mucous membrane appearing blistered in all animals after 1 h Slightly irritating

NZW = New Zealand White

Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.

For details regarding studies reviewed during the 2001 EU evaluation please refer to the Monograph.

Reference: IIA, 5.2.5.701

Report: [REDACTED] 2007, Glyphosate Technical (NUP 05068): Primary Eye Irritation Study In Rabbits

[REDACTED] Study No.: B02305

Data owner: Nufarm

Date: 2007-03-06, unpublished

ASB2012-11428

Guidelines: OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004), JMAFF guideline 2-1-5 (2005)

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate technical

Identification: NUP 05068

Description: Solid

Lot/Batch #: 200609062
Purity: 95.1 %
Stability of test compound: Stable under storage conditions (20 ± 5 °C), light protected;
Expiry date: 2008-09-14
Vehicle and/or positive control: None
Test animals:
Species: Rabbit
Strain: New Zealand White, SPF
Source: Harlan Netherlands BV, NL-5961 NM Horst, and NL-5960
Age: 15 weeks (male); 12 and 15 weeks (females)
Sex: One male and 2 females
Weight at dosing: 2.969 kg (male), 2.605 kg and 3.416 kg (females)
Acclimation period: At least five days
Diet/Food: Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugst), *ad libitum*
Water: Tap water, *ad libitum*
Housing: Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blocks and haysticks were provided for gnawing.
Environmental conditions: Temperature: 17 - 23 °C
Humidity: 30 - 70 %
Air changes: 10 - 15/hour
12 hours light/dark cycle

Study design and methods:

In life dates: 2007-01-17 to 2007-02-26

Animal assignment and treatment:

The test was conducted using young adult New Zealand albino rabbits (1 male, 2 females). The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the left eye of the rabbits. The lids were then gently held together for about one second. The treated eyes were not rinsed after instillation. The right eye remained untreated and served as the reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after instillation. Scleral reddening and ocular discharge was also assessed. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.

Results and discussion

Mortality: No mortality occurred.

Clinical observations: No clinical signs of systemic toxicity were observed during the study.

Body weight: All rabbits showed the expected body weight gain.

Necropsy: No necropsy was performed.

Eye observations: Very slight to slight corneal opacity were observed in all rabbits from 1 hour after instillation up to 72 hours. No signs of iritis, corrosion or staining were observed in any animal throughout the study period. One hour after instillation slight to moderate conjunctival redness was observed in the treated eyes of all rabbits. By 24 hours the redness increased to marked in two animals and to moderate in one rabbit. After 48 hours the conjunctival redness decreased in all rabbits. Only slight redness was observed in the rabbits after 7 days. Moderate to marked chemosis of the conjunctivae was observed from 1 hour after instillation up to 24 hours. The swelling decreased with time. 72 hours after treatment slight swelling was still present in two animals. One hour after instillation two rabbits exhibited moderate ocular discharge. Moderate or marked discharge was observed in all animals at the 24-hour reading time point. This persisted at the 48-hour reading as slight or moderate in all rabbits. After 72 hour slight discharge was still present in one rabbit.

Reddening of the sclera was observed in all animals. However, one hour after instillation sclera of one animal was not assessable due to conjunctival swelling. In two animals moderate or marked reddening of the sclera was observed at this time point. After 24 hours all rabbits showed marked reddening of the sclera. This sign persisted in the rabbits as moderate or marked at the 48- and 72-hour readings. In one animal slight reddening was still present after 7 days. All rabbits were free of ocular signs by day 10 after instillation.

The group mean irritation scores (24 to 72 hours) were calculated to be 1.5 for corneal opacity, 0.0 for iris lesions, and 2.2 conjunctival redness, and 1.7 for chemosis of the conjunctiva.

The individual scores for each time point, individual mean and group mean scores (24 to 72 hours) are presented in Table B.6.2-26.

Table B.6.2-26: Results of the eye irritation test

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		Sclera
		Opacity	Area		Redness	Chemosis	
Rabbit 1 (male)	1	1	1	0	2	3	3
	24	2	2	0	3	3	3
	48	2	2	0	3	2	3
	72	1	1	0	2	1	2
	Day 7	0	0	0	1	0	1
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
Mean (24, 48, 72 h)		1.67	---	0.0	2.67	2.0	---
Rabbit 2 (female)	1	1	1	0	1	2	2
	24	2	4	0	2	3	3
	48	2	4	0	2	2	3
	72	2	4	0	2	1	2
	Day 7	0	0	0	1	0	0
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	0.0	2.0	2.0	---
Rabbit 3 (female)	1	1	4	0	2	3	n.a.
	24	1	4	0	3	2	3
	48	1	4	0	2	1	2
	72	0	0	0	1	0	2
	Day 7	0	0	0	1	0	0
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
Mean (24, 48, 72 h)		0.67	--	0.0	2.0	1.0	---
Group means		1.5	---	0.0	2.2	1.7	---

n.a. = not assessable due to swelling of the conjunctivae

Conclusion by the Notifiers

Based on the study results and on the and according to EU classification criteria the test substance glyphosate technical (NUP 05068) is to be classified as irritating to the eyes (Xi, R36). This corresponds to Category 2A according to the OECD Globally Harmonized System (GHS) classification criteria.

Comment by RMS:

The study is considered acceptable and the results revealed that glyphosate acid is irritant to rabbit eyes..

Reference: IIA, 5.2.5/02

Report: [REDACTED] 2009, Acute Eye Irritation/Corrosion Test Of Glyphosate TC In Rabbits

[REDACTED]
Data owner: Helm AG

Report No.: 24878

Date: 2009-11-27, unpublished

ASB2012-11429

Guidelines: OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004), OPPTS 870.2400 (1998)

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:

Identification: Glyphosate TC

Description: White powder

Lot/Batch #: 2009051501

Purity: 96.4 %

Stability of test compound: At room temperature in the dark stable until May 15, 2011.

Vehicle and/or positive control: None

Test animals:

Species: Rabbit

Strain: Himalayan

Source: [REDACTED]

Age: Approx. 6.5 - 7.5 months

Sex: Males

Weight at dosing: 2.5 - 2.8 kg

Acclimation period: At least 20 days.

Diet/Food: ssniff K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period
 Water: Tap water, *ad libitum* before and after the exposure period
 Housing: Individual housing
 Environmental conditions: Temperature: 20 ± 3 °C
 Humidity: 30 - 70 %
 Air changes: no data
 12 hours light/dark cycle

Study design and methods

In life dates: 2009-10-15 to 2009-10-29

Animal assignment and treatment:

The test was conducted using three young male adult Himalayan albino rabbits. The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the right eye of the rabbits. The lids were then gently held together for about one second. 1 hour after instillation the eyes were rinsed with 20 mL NaCl solution. The left eye remained untreated and served as the reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours, as well as 4, 5, 6, 7 and 8 days after instillation. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of the study and at termination.

Results and discussion

Mortality: No mortality occurred.

Clinical observations: No clinical signs of systemic toxicity were observed during the study.

Body weight: There were no effects on body weight noted.

Necropsy: No necropsy was performed.

Eye observations: Corneal opacity (grade 1) was observed in all animals 24 to 72 hours, in animal no. 2 until 4 days and in animal no. 1 until 7 days after instillation. The fluorescein test performed 24 hours after instillation demonstrated corneal staining in all animals. The fluorescein test performed 7 days after instillation demonstrated corneal staining only in animal no. 1.

Irritation of the iris (grade 1) was observed in all animals 24 hours, in animal no. 2 until 48 hours and in animal no. 1 until 72 hours after instillation.

Conjunctival redness (grade 1) was observed in all animals 60 minutes to 72 hours, in animal no. 1 until 4 days and in animal no. 2 until 5 days after instillation.

Chemosis (grade 1) was observed in all animals 60 minutes, in animal no. 2 until 24 hours and in animal no. 1 until 48 hours after instillation.

In addition, secretion was observed in all animals 60 minutes after instillation. There were no systemic intolerance reactions. All rabbits were free of ocular signs by Day 8 after instillation. The group mean irritation scores (24 to 72 hours) were calculated to be 1.0 for corneal opacity, 0.7 for iris lesions, and 1.0 conjunctival redness and 0.3 for chemosis of the conjunctiva.

The individual scores for each time point, individual mean and group mean scores (24 to 72 hours) are presented in Table B.6.2-27.

Table B.6.2-27: Results of the eye irritation test

Animal	Scoring [h]	Cornea	Iris	Conjunctiva	
				Redness	Chemosis
Rabbit 1	1	0	0	1	1
	24	1	1	1	1
	48	1	1	1	1
	72	1	1	1	0
	Day 4	1	0	1	0
	Day 5	1	0	0	0
	Day 6	1	0	0	0
	Day 7	1	0	0	0
Mean (24, 48, 72 h)		1.0	1.0	1.0	0.7
Rabbit 2	1	0	0	1	1
	24	1	1	1	1
	48	1	1	1	0
	72	1	0	1	0
	Day 4	1	0	1	0
	Day 5	0	0	1	0
	Day 6	0	0	0	0
	Day 7	—	—	—	—
Mean (24, 48, 72 h)		1.0	0.7	1.0	0.3
Rabbit 3	1	0	0	1	1
	24	1	1	1	0
	48	1	0	1	0
	72	1	0	—	0
	Day 4	0	0	0	0
	Day 5	—	—	—	—
	Day 6	—	—	—	—
	Day 7	—	—	—	—
Mean (24, 48, 72 h)		1.0	0.3	1.0	0.0
Group means		1.0	0.7	1.0	0.3

Conclusion by the Notifiers

Based on the scores for cornea, iris and conjunctiva and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, glyphosate TC is not to be classified for eye irritation.

Comment by RMS:

The study is considered supplementary: Based on the study design, it is not possible to conclude, that the test substance is not irritating to rabbit eyes, because the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405. The results provided only additional information.

Reference:

IIA, 5.2.5/03

Report:

1995, HR-001: Primary Eye irritation study in rabbits.

Report No.: IET 95-0034

Data owner: Arysta LifeScience

Date: 1995-06-29, unpublished

ASB2012-11430

Guidelines:

U.S. EPA FIFRA Guideline Subdivision F

MAFF 59 NohSan No. 4200 (1985)

Materials and methods

Study design and methods:

Animal assignment and treatment:

Results and discussion

Mortality: No mortality occurred.

Clinical observations: No clinical signs of systemic toxicity were observed during the study.

Body weight: All rabbits showed the expected body weight gain.

Necropsy: No necropsy was performed.

Eye observations: Results of the no eye irrigating group are summarised in the Table B.6.2-28.

Without eye irrigation

Irritation of cornea

At 1 hour after application, all animals showed score 2, the easily discernible translucent area, details of iris slightly obscured. At 24 hours after application, one animal showed score 3, nacreous area, no details of iris visible, size of pupil barely discernible. These opacities remained until Day 21 in three animals.

Irritation of iris

At 1 hour after application, all animals showed score 1, the congestion and/or markedly deepened rugae of iris. The irritation disappeared by Day 10.

Irritation of conjunctivae

At 1 hour after application, all groups showed redness score 1, the definite hyperemia of some blood vessels. At 24 or 48 hours after application, score 2 redness of conjunctivae in all animals. These conjunctival irritations gradually began to decrease thereafter, and disappeared by Day 16.

At 1 hour after application, 4 animals showed chemosis score 2, the obvious swelling with partial eversion of lids. In addition, 2 animals showed score 3, the swelling with lids about half closed. The chemosis disappeared by Day 7.

With eye irrigation (30 seconds or 2 minutes after application)

The irridial and conjunctival irritations observed in irrigation group were almost the same as those in non-irrigating group, while the corneal irritation was slighter.

Animals in the irrigating groups showed reduced eye irritations and faster recovery as compared with animals of the non-irrigating group. Each of irrigation at 30 seconds or 2 minutes after application was effective for reduction of irritation and for recovery.

Table B.6.2-28: Results of the eye irritation without eye irrigation after application*

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1 (female)	1	2	4	1	1	2	3
	24	2	4	1	2	2	3
	48	2	4	1	2	2	3
	72	2	4	1	2	2	1
	Day 4	1	3	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1	1	0	1	0	0
	Day 13	1	1	0	1	0	0
	Day 16	1	1	0	0	0	0
	Day 19	1	1	0	0	0	0
	Day 21	1	1	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	1.0	2.0	2.0	---

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 2 (female)	1	2	4	1	1	2	3
	24	3	4	1	2	2	3
	48	3	4	1	2	2	3
	72	2	3	1	2	1	1
	Day 4	2	2	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1	2	0	1	0	0
	Day 13	1	2	0	1	0	0
	Day 16	1	2	0	0	0	0
	Day 19	1	2	0	0	0	0
	Day 21	1	2	0	0	0	0
Mean (24, 48, 72 h)		2.67	---	1.0	2.0	1.67	---
Rabbit 3 (female)	1	2	4	1	1	3	3
	24	2	4	1	2	3	3
	48	2	4	1	2	2	3
	72	2	4	1	2	2	3
	Day 4	2	3	1	2	1	0
	Day 7	1	2	1	1	0	0
	Day 10	1	2	0	1	0	0
	Day 13	1	2	0	1	0	0
	Day 16	1	2	0	0	0	0
	Day 19	1	1	0	0	0	0
	Day 21	1	1	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	1.0	2.0	2.33	---
Rabbit 4 (female)	1	2	4	1	1	3	3
	24	2	4	1	2	3	3
	48	2	4	1	2	2	3
	72	2	3	1	2	2	1
	Day 4	2	2	1	2	1	1
	Day 7	1	2	0	1	0	0
	Day 10	1	2	0	1	0	0
	Day 13	1	2	0	1	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	1.0	2.0	2.33	---
Rabbit 5 (female)	1	2	4	1	1	2	3
	24	2	4	1	2	2	3
	48	2	4	1	2	2	3
	72	2	4	1	2	2	1
	Day 4	1	2	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1	1	0	1	0	0
	Day 13	0	0	0	0	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Mean (24, 48, 72 h)		2.0	---	1.0	2.0	2.0	---

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 6 (female)	1	2	4	1	1	2	3
	24	2	4	1	2	2	3
	48	2	2	1	2	2	2
	72	1	2	0	2	1	0
	Day 4	1	1	0	1	0	0
	Day 7	0	0	0	0	0	0
	Day 10	0	0	0	0	0	0
	Day 13	0	0	0	0	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Mean (24, 48, 72 h)		1.67	---	0.67	2.0	1.67	---
Group means		2.1	---	1.0	2.0	2.0	---

* - according to the report

Table B.6.2-29: Results of the eye irritation with eye irrigation (30 seconds after application)*

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 7 (female)	1	0	0	0	1	3	2
	24	1	3	1	1	2	3
	48	1	1	0	2	2	1
	72	1	1	0	1	1	1
	Day 4	0	0	0	1	0	0
	Day 7	0	0	0	0	0	0
Mean (24, 48, 72 h)		1.0	---	0.33	1.33	1.67	---
Rabbit 8 (female)	1	0	0	0	1	2	2
	24	1	2	0	1	2	2
	48	1	1	0	1	1	1
	72	0	0	0	1	0	0
	Day 4	0	0	0	1	0	0
	Day 7	0	0	0	0	0	0
Mean (24, 48, 72 h)		0.67	---	0.0	1.0	1.0	---
Rabbit 9 (female)	1	1	2	1	1	2	2
	24	1	3	1	2	2	3
	48	1	3	0	2	1	1
	72	1	2	0	1	1	1
	Day 4	0	0	0	1	0	0
	Day 7	0	0	0	0	0	0
Mean (24, 48, 72 h)		1.0	---	0.33	1.67	1.33	---
Group means		0.9	---	0.2	1.3	1.3	---

* - according to the report

Conclusion by the Notifiers

Based on the study results and on the and according to EU classification criteria the test substance glyphosate technical (HR-001) is to be classified as irritating to the eyes (Xi, R36). This corresponds to Category 2 according to the OECD Globally Harmonized System (GHS) classification criteria.

Comment by RMS:

The study is considered acceptable. The test substance was irritating to rabbit eyes.

Reference: IIA, 5.2.5/04
Report: [REDACTED] 2009, Expert Statement Glyphosate technical: Primary eye irritation study in rat
[REDACTED] Data owner:
Excel
Report No.: C22897
Date: 2009-01-23, not published
ASB2012-11431
Guidelines: OECD 405 (2002)
Council Regulation (EC) No 440/2008 (2008)
Deviations None
GLP: yes
Acceptability: Please see comment by RMS

Executive Summary

A pH measurement was performed with the test item in a 1% (w/w) solution in purified water before the study initiation. The pH of the test item was found to be 1.93.

According to the OECD Guidelines 405 and Council Regulation (EC) No 440/2008 B.5:

Physicochemical properties and chemical reactivity – Substances exhibiting pH extremes such as $\leq 2,0$ may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive or irritant to the eye, then its acid reserve (buffering capacity) may also be taken into consideration.

It is assumed that the test substance item has corrosive properties; therefore, no eye irritation study in rabbits with Glyphosate Technical was performed.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate technical is classified for eye irritation:

- Xi R41 (Risk of serious damage to eyes);
- Category 1, H318 (Causes serious eye damage).

Conclusion by the Notifiers

The eye irritation of the test material (glyphosate technical) was concluded to be positive. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate technical is classified for eye irritation: Category 1, H318 (Causes serious eye damage).

Comment by RMS:

The study is considered acceptable. Agreed on the statement provided by the Notifiers.

Reference: IIA, 5.2.5/05
Report: [REDACTED] 2009, Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits
[REDACTED]
Data owner: Helm AG
Report No.: LPT 23914
Date: 2009-04-30, unpublished
ASB2012-11432
Guidelines: OECD 405 (2002)
US EPA OPPTS 870.2400.

Deviations: Personnel change of the head of the Quality Assurance Unit:
until January 31, 2009: Dipl. Biol. S. Steuer
as of February 1, 2009: Dr. med. vet. habil. K. R. Sultan. This minor deviation did not have any effect on the validity and integrity of the scientific results obtained in this study.

GLP yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material: Glyphosate
Identification: Glyphosate TC
Description: White powder
Lot/Batch #: 20080801
Purity: 98.8 %
Stability of test compound: No data given in the report.
Vehicle and/or positive control: No vehicle used
Test animals:
Species: Rabbit
Strain: Himalayan
Source: [REDACTED]
Age: Approx. 4 - 32.5 months
Sex: Male
Weight at dosing: Animal no. 1 : 4.0 kg
Animal no. 2: 3.9 kg
Animal no. 3: 4.1 kg
Acclimation period: At least 20 adaptation days
Diet/Food: Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available ad libitum before and after the exposure period.
Water: Tap water, *ad libitum*
Housing: The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany).
Environmental conditions: Temperature: 20 ± 3 °C
Humidity: 30 - 70 %
12-hour light/dark cycle

Study design and methods

In life dates: 2009-02-02 to 2009-02-15

Animal assignment and treatment:

100 mg of the test item was administered into one eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control. The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, 2 further animals were employed 24 hours after start of the initial test.

1 hour after instillation the eyes were rinsed with 20 mL NaCl solution. The eyes were examined ophthalmoscopically with a slit lamp prior to the administration and 1, 24, 48, 72 hours and 4 days after the administration. The eye reactions were observed and registered. 24 hours after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: A single instillation of 100 mg glyphosate TC per animal into the conjunctival sac of the right eye of three rabbits caused the following changes:

Corneal opacity (grade 1) was observed in animal no. one 24 to 72 hours and in animal no. three 24 and 48 hours after instillation.

The fluorescein test performed 24 hours after instillation revealed corneal staining in animal no. 1 and 3 (up to 1/4 of the surface).

Conjunctival redness (grade 1) was observed in all animals 60 minutes to 48 hours, in animal no. 1 until 72 hours after instillation.

Chemosis (grade 1) was observed in animal no. one 24 and 48 hours after instillation.

In addition, secretion was observed in all animals 60 minutes after instillation.

The irises were not affected by instillation of the test item.

There were no systemic intolerance reactions.

Conclusion by the Notifiers

According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions Glyphosate TC was non – irritating to eyes, hence, no labelling is required.

Comment by RMS:

The study is considered supplementary: Based on the study design, it is not possible to conclude, that the test substance is not irritating to rabbit eyes, because the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405. The results provided only additional information.

Reference:

IIA, 5.2.5/06

Report:

2010, Acute Eye Irritation/Corrosion Test of
Glyphosate TC in Rabbits

Data owner: Helm AG

Report No.: LPT 24606

Guidelines: Date: 2010-01-06, unpublished
ASB2012-11433
OECD 405 (2002)
US EPA OPPTS 870.2400.

Deviations: No deviations from the Study Plan.

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material: Glyphosate

Identification: Glyphosate TC

Description: White powder

Lot/Batch #: 20090506

Purity: 97.3 %

Stability of test compound: No data given in the report.

Vehicle and/or positive control: No vehicle was used

Test animals:

Species: Rabbit

Strain: Himalayan

Source: LPT Laboratory of Pharmacology
and Toxicology GmbH & Co. KG
Branch Löhndorf
24601 Löhndorf/Post Wankendorf, Germany

Age: Approx. 6 - 8 months

Sex: Male

Weight at dosing: At dosing
Animal no. 1 : 2.5 kg
Animal no. 2: 2.5 kg
Animal no. 3: 2.7 kg

Acclimation period: At least 20 adaptation days

Diet/Food: Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available *ad libitum* before and after the exposure period.

Water: Tap water, *ad libitum*

Housing: The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany).

Environmental conditions: Temperature: 20 ± 3 °C
Humidity: 30 - 70 %
12-hour light/dark cycle

Study design and methods

In life dates: 2009-10-26 to 2009-11-12

Animal assignment and treatment:

100 mg of the test item were administered into one eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control. The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, 2 further animals were employed 24 hours after start of the initial test.

1 hour after instillation the eyes were rinsed with 20 mL NaCl solution. The eyes were examined ophthalmoscopically with a slit lamp prior to the administration and 1, 24, 48, 72 hours and 4 to 7 days after the administration. The eye reactions were observed and registered.

24 hours and 7 days after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Corneal opacity (grade 1) was observed in all animals 24 to 72 hours, in animal no. 1 until 4 days and in animal no. 3 until 5 days after instillation. The fluorescein test performed 24 hours after instillation revealed corneal staining in all animals (1/2 to 3/4 of the surface). Irritation of the iris (grade 1) was observed in all animals 24 and 48 hours, in animal no. 3 until 72 hours after instillation. Conjunctival redness (grade 1 or 2) was observed in all animals 60 minutes to 4 days, in animal no. 3 until 6 days after instillation. Chemosis (grade 1) was observed in all animals 60 minutes and 24 hours after instillation. In addition, secretion was observed in all animals 60 minutes and 24 hours after instillation. There were no systemic intolerance reactions.

Conclusion by the Notifiers

According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions Glyphosate TC was non - irritating to eyes, hence, no labelling is required.

Comment by RMS:

The study is considered supplementary: Based on the study design, it is not possible to conclude, that the test substance is not irritating to rabbit eyes, because the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405. The results provided only additional information.

Reference:

IIA, 5.2.5/07

Report:

[REDACTED] 2009, Glyphosate – Acute Eye Irritation Study in Rabbits

Data owner: Helm AG

Report No.: 12172-08

Date: 2009-03-11, unpublished

ASB2012-11434

Materials and methods

Study design and methods

Animal assignment and treatment:

Healthy albino rabbits were released from quarantine. Both eyes of each animal were carefully examined within 24 hours prior to treatment with a fluorescein sodium ophthalmic solution and cobalt-filtered light. Both eyes of each animal were again carefully examined just prior to treatment, but without the fluorescein sodium ophthalmic solution. Only those animals without eye defects or irritation were selected for testing. On Day 0, a dose of 0.1 mL by volume (93.2 mg) of the undiluted test substance was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one second to prevent loss of material. The untreated left eyes served as comparative controls.

The grades of ocular reaction were recorded at 1, 24, 48 and 72 hours, and at 4, 7, 10, 14 and 17 days after treatment. The corneas of all treated eyes were examined immediately after the 24 hour observation with a fluorescein sodium ophthalmic solution. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The maximum average irritation score of 3 1.7, obtained at 24 hours after treatment, was used to rate

Glyphosate moderately irritating. Fluorescein staining was observed in two of three eyes at 24 hours after treatment and was not observed in any eyes on Day 10 after treatment. Toxicity categories are determined by the presence and duration of corneal involvement, iridic irritation, and positive conjunctival irritation. Any corneal involvement or iridic irritation with a score of 1 or more is considered positive. Any conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive.

Conclusion by the Notifiers

Based on the maximum average irritation score of 3 1.7, the test substance Glyphosate is rated moderately irritating. Since all positive effects had cleared on Day 10 after dosing, the test substance is assigned to Toxicity Category 11. No irritation was observed in any eyes on Day 17.

Comment by RMS:

The study is considered acceptable and the test substance is irritating to rabbit eyes. The Notifiers conclusion on irritating potential based on US EPA criteria: Toxicity Category II (not 11, obviously typing error) due to average irritation score of 31.7 in rabbit eyes

Reference:

IIA, 5.2.5/08

Report:

2005, Eye Irritation/Corrosion Effects in rabbits (Oryctolagus cuniculus) of Glyphosate 95 TC

Data owner: Helm AG

Report No.: PSL 15277

Date: 2005-04-04, unpublished

ASB2012-11435

Guidelines:

US EPA OPPTS 870.2400

OECD 405 (2002).

Deviations:

No deviations from Study Plan.

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Glyphosate

Identification:

Glyphosate Acid Technical

Description:

White crystalline powder

Lot/Batch #:

040205

Purity:	97.23 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	No vehicle was used.
Test animals:	
Species:	Rabbit
Strain:	New Zealand albino
Source:	
Age:	Young adult
Sex:	Male
Weight at dosing:	No data given in the report
Acclimation period:	7 days
Diet/Food:	Pelleted Purina Rabbit Chow #5326
Water:	Tap water, <i>ad libitum</i>
Housing:	The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent <i>Guide for the Care and Use of Laboratory Animals DHEW (NIH)</i> . Litter paper was placed beneath the cage and was changed at least three times per week
Environmental conditions:	Temperature: 18-22 °C 12-hour light/dark cycle

Study design and methods:

In life dates: 2004-05-26 to 2004-06-05

Animal assignment and treatment:

A primary eye irritation test was conducted with rabbits to determine the potential for Glyphosate Acid Technical to produce irritation from a single instillation via the ocular route. Under the conditions of this study, the test substance is classified as severely irritating to the eye. Prior to use, the test substance was ground to a powder. One-tenth of a milliliter (0.06 grams) of the ground test substance was instilled into the right eye of three healthy rabbits. The left eye remained untreated and served as a control. Ocular irritation was evaluated by the method of Draize *et al.*. One hour after test substance instillation, all three treated eyes exhibited corneal opacity, iritis, and conjunctivitis. The overall incidence and severity of irritation decreased gradually over time. All animals were free of ocular irritation by Day 10 (study termination).

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: All animals appeared active and healthy. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. One hour after test substance instillation, all three treated eyes exhibited corneal opacity, iritis, and conjunctivitis. The overall incidence and severity of irritation decreased gradually over time. All animals were free of ocular irritation by Day 10 (study termination).

Conclusion by the Notifiers

Under the conditions of this study, Glyphosate Acid Technical is classified as severely irritating to the eye.

Comment by RMS:

The study is considered acceptable and the conclusion by the Notifiers is supported.

Reference: IIA, 5.2.5/09

Report: [REDACTED] 2008, Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical [REDACTED]

Data owner: Helm AG

Report No.: RF-3996.312.599.07

Date: 2008-09-12, unpublished

ASB2012-11436

Guidelines: OECD 405 (2002).

Deviations: The experimental phase initiation and experimental phase conclusion dates were updated.

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate
Identification: Glyphosate Technical
Description: White powder
Lot/Batch #: 20070606
Purity: 98.95 %
Stability of test compound: No data given in the report.
Vehicle and/or positive control: No vehicle was used
Test animals:
Species: Rabbit
Strain: New Zealand White
Source: [REDACTED]
Age: 18 weeks old
Sex: One male and one female
Weight at dosing: between 3.346 and 3.624 kg
Acclimation period: 5 to 6 days
Diet/Food: Pelleted and autoclaved commercial diet for rabbits (*Guabi, Mogiana Alimentos S.A. - Brazil*)
Water: Tap water, *ad libitum*
Housing: The animals were housed individually in galvanised steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.

Environmental conditions: Temperature: 17 - 22 °C
 Humidity: 30 - 70 %
 Air changes: 10 - 15/hour
 12-hour light/dark cycle

Study design and methods:

In life dates: 2008-05-26 to 2008-06-17

Animal assignment and treatment:

0.1 g of the test item was applied to the eye of each animal. The test item was applied into the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. Following application, the eyelids were gently held together for about one second in order to prevent test item loss. The right eye that remained untreated was used as a negative control.

The test was performed initially using one animal for evaluation of any irritant/corrosive effect of the test item to the eye. Because some severe ocular reactions were observed in the initial test, only one additional animal was tested to confirm the response.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The following additional ocular changes were noted to the animals during the observation period:

Rabbit #04: Blepharitis at the 48- and 72-hr time points.

Rabbit #05: Blepharitis at the 24-, 48- 72-hr, and 7- and 14-day time points; and a small raised off area on the corneal surface in the right inferior quadrant at the 21-day time point.

Conclusion by the Notifiers:

Based on the "UN GHS / EU CLP Criteria for Classification (UN, 2009; EC, 2008)" the ocular Classification for Glyphosate Technical is "Category I Irreversible effects on the eye".

Comment by RMS:

The study is considered acceptable and the conclusion by the Notifiers is supported.

Reference: IIA, 5.2.5/10

Report: [REDACTED] 1988, Primary Eye Irritation Study of
 Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White
 Rabbits

[REDACTED]
 Data owner: Monsanto
 Monsanto Report No.: FD-88-29
 Date: 1988-06-08, unpublished
 Z35395

Guidelines: US EPA 81-4

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Stability of test compound:	Stored at room temperature
Vehicle and/or positive control:	None
Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	
Age:	Young adult
Sex:	Not specified
Weight at dosing:	Between 2-3 kg
Acclimation period:	At least five days
Diet/Food:	NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Gardners, PA, US), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	Individually in wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 °C Humidity: 40 – 60 % Air changes: Not specified Light cycle: 12 hours light/dark cycle

Study design and methods

In life dates: 1988-04-11 to 1988-05-02

Animal assignment and treatment:

The test was conducted using six young adult New Zealand albino rabbits. The test substance (0.1 g) was instilled into one eye of each rabbit. The lower eyelid was pulled gently away from the eyeball to form a cup (conjunctival sac) and the test substance inserted therein. The lids were then held together for one second and released. Following scoring at 24 hours after dose administration, any residual material was rinsed from the eye with physiological saline. Treated and untreated eyes were examined at 1, 24, 48, and 72 hours, and 7, 14, and 21 days after test substance instillation. The cornea, iris, and conjunctiva were scored separately according to the Draize system. The animals were observed twice daily for mortality at least five hours apart. Body weights were obtained on study day 1 prior to dose administration and at death. At study termination, surviving animals were euthanized by intracardiac injection of sodium pentobarbital and discarded.

Results and discussion

Mortality: One rabbit was found dead 20 days after dose administration. Prior to death, this animal exhibited anorexia, and gross necropsy revealed a clear gel-like substance in the large intestine. These findings are consistent with mucoid enteropathy, a condition occasionally

noted in stock laboratory rabbits. Therefore, the death was considered spontaneous and unrelated to treatment.

Clinical observations: Not reported.

Body weight: Not reported.

Necropsy: Not reported.

Eye observations: At one hour after test substance instillation, all animals exhibited conjunctival irritation (redness, swelling, blistering and discharge). Corneal opacity was noted one hour after test substance instillation in four of six (4/6) animals. Corneal opacity and conjunctival irritation were noted in all rabbits at the 24, 48 and 72 hour and 7 day examinations. Three rabbits exhibited pannus on the cornea; two eyes (iris) had sluggish reactions to light; one rabbit had prominent vascularization of the conjunctival and another animal had a blood-like discharge. Corneal opacity persisted through study termination (day 21) in three of five (3/5) rabbits. Of the remaining two rabbits, one exhibited slight conjunctival discharge at study termination and the other rabbit's treated eye appeared normal 14 days after dose administration.

The group mean irritation scores (24 to 72 hours) were calculated to be 2.1 for corneal opacity, 0.2 for iris lesions, 2.0 for conjunctival redness, and 2.6 for conjunctival chemosis. The individual scores for each time point, individual mean and group mean scores (24 to 72 hours) are presented in Table B.6.2-30.

Table B.6.2-30: Results of the Eye Irritation Test ^a

Animal No.	Scoring [h]	Cornea		Iris	Conjunctivae		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1	1	2	1	0	2	2 ^b	2
	24	2	3	0	2	2 ^b	2
	48	3	1	0	2	2 ^b	2 ^c
	72	3 ^d	1	0	2	2 ^b	2
	7 days	3 ^d	1	0	2	1	0
	14 days	2		0	1	0	0
	21 days	2	1	0	0	0	0
Mean	24-72 h	2.7		0.0	2.0	2.0	
Rabbit 2	1	2	1	0	2	2 ^b	3
	24	2	2	0	2	4 ^b	3
	48	2	2	0	2	4 ^b	2
	72	1	1	0	2	2 ^b	1
	7 days	2	1	0	1	1	0
	14 days	2	1	0	0	0	1
	21 days	0	0	0	0	0	1
Mean	24-72 h	1.7		0.0	2.0	3.3	
Rabbit 3	1	2	1	0	2	2 ^{b,e}	2
	24	2	2	1	2	4 ^{b,e}	2
	48	2	1	1	2	3 ^{b,e}	2
	72	2	1	1	2	3 ^{b,e}	2
	7 days	3 ^d	2	1	3	2 ^b	2
	14 days	2	1	1	1	0	0
	21 days	2	1	0	0	0	0
Mean	24-72 h	2.0		1.0	2.0	3.3	

WARNING: This document forms part of an ECV evaluation data package and should not be used in isolation. Registration must not be granted on the basis of this document.

Animal No.	Scoring [h]	Cornea		Iris	Conjunctivae		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 4	1	0	0	0	2	3 ^b	3
	24	1	4	0	2	4 ^b	3
	48	1	3	0	2	2 ^b	2
	72	1	3	0	2	2 ^b	2
	7 days	3	1	0	2	2	1
	14 days	0	0	0	0	0	0
	21 days	0	0	0	0	0	0
Mean	24-72 h	1.0		0.0	2.0	2.7	
Rabbit 5	1	0	0	0	2	2 ^b	2
	24	2	2	0	2	2 ^b	2
	48	2	2	0	2	2 ^b	2
	72	3	1	0	2	2 ^b	1
	7 days	4 ^d	1	1	2	2	1
	14 days	3 ^d	1	1	2	1	0
	21 days	3 ^d	1	0	1	0	0
Mean	24-72 h	2.3		0.0	2.0	2.0	
Rabbit 6	1	2	1	0	2	2 ^b	3
	24	2	2	0	2	2 ^b	2
	48	3	1	0	2	2 ^b	2
	72	3	1	0	2	2 ^b	0
	7 days	2	1	0	1	1	0
	14 days	2	1	0	0	0	0
	21 days	- ^f	-	-	-	-	-
Mean	24-72 h	2.7		0.0	2.0	2.0	
Group mean	24-72 h	2.1		0.2	2.0	2.6	

^a Scores for treated eyes; untreated eyes appeared normal at all times.

^b Mucus membrane of the eyelid appeared blistered.

^c Blood-like discharge noted.

^d Pannus on the cornea.

^e Prominent vascularisation of the conjunctiva.

^f Animal found dead 20 days after dose administration.

Conclusion by the Notifiers

Based on the study results and on the and according to EU classification criteria the test substance glyphosate is to be classified as risk of serious damage to the eyes (Xi, R41). This corresponds to Category 1 according to the OECD Globally Harmonized System (GHS) classification criteria.

Comment by RMS:

The study is considered acceptable and the conclusion by the Notifiers is supported.

Reference:

IIA, 5.2.5/11

Report:

1997 Glyphosate Acid: Eye Irritation to the Rabbit

Data owner: Syngenta

Report No.: /P/5138

Date: 1997-03-18, not published

TOX2000-1986

Guidelines:

OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2004)

Deviations: None.
GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6 % w/wi
CAS#:	Not reported
Stability of test compound:	The test substance was used within the expiry date
Vehicle and/or positive control:	None
Test Animals:	
Species	Rabbit
Strain	New Zealand White albino
Age/weight at dosing	Young adult / 2951-3702 g
Source	
Housing	Individually in aluminium sheet cages in racks suitable for animals of this strain and the weight range expected during the course of the study
Acclimatisation period	At least 6 days
Diet	STANRAB SQC, (Special Diet Services Limited, Stepfield, Witham, Essex, UK) <i>ad libitum</i>
Water	Mains water <i>ad libitum</i>
Environmental conditions	Temperature: 17±2 °C Humidity: 40-70 % Air changes: Approximately 25/hour Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 22 May 1996 End: 6 July 1996

Animal assignment and treatment: Initially, the test substance (approximately 100mg) was applied into the conjunctival sac of the left eye of one rabbit by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were then gently held together for 1-2 seconds after which the animal was released. The other eye was untreated (control eye).

When the eye irritation potential had been fully assessed in the first animal, the test substance was applied into the test eye of the remaining five animals, as described previously.

As the initial pain reaction of the first rabbit was moderate and the irritation was less than severe, the eyes of the remaining rabbits were pre-treated with five drops of local anaesthetic (OPHTHAINE, 0.5% proparacaine hydrochloride solution) at three minute intervals between each drop.

Both eyes of each rabbit were examined within the twenty-four hours prior to dosing. The examination consisted of a visual assessment with the aid of fluorescein and only rabbits without any apparent eye defects or ocular irritation were used.

Immediately after the application of the test substance, an assessment of the initial pain reaction of the rabbit was made using a six-point scale.

The eyes were examined and the Draize scale was used to assess the grade of ocular reaction approximately one hour and 1, 2, 3, 4, 7 and 8 days after application where necessary. In addition, as an aid in the assessment of corneal damage, fluorescein staining was used at all readings from 1 day after application. A modified form of the Kay and Calandra system was used to interpret and classify the numerical scores.

Results and discussion

No deaths occurred. No systemic signs of toxicity were noted during the study.

Application into the eye caused moderate initial pain in the first animal dosed, therefore the subsequent five animals were pre-treated with the local anaesthetic OPHTHAINE prior to dosing. The group initial pain reaction was none to moderate (class 0-3 on a 0-5 scale).

Corneal effects, consisting of slight to mild opacity affecting up to the entire cornea, were seen in all animals during the first two days, persisting to day 4 in five rabbits. Slight iritis was seen in all animals during the first two days, persisting to day 3 in two rabbits. Conjunctival, effects consisting of slight to moderate redness, slight to mild chemosis and slight to severe discharge, were seen in all animals up to day 4.

Additional observations included mucoid discharge, eye closed, irregular corneal surface, convoluted eyelids, erythema of the upper and/or lower eyelids, raised corneal opacity, Harderian gland discharge and nictitating membrane partially haemorrhagic.

All signs of irritation had completely regressed in five animals 7 days after application. Slight conjunctival redness was seen in the remaining animal on day 7; the animal had completely recovered by day 8.

Table B.6.2-31: Eye irritation scores of glyphosate acid (95.6 % w/w) according to the Draize scheme

Time		Cornea						Iris						Conjunctiva																	
														Redness						Chemosis											
Animal number		17	18	19	7	8	9	17	18	19	7	8	9	17	18	19	7	8	9	17	18	19	7	8	9	17	18	19	7	8	9
after 1 hour		0	0	0	1	1	1	0	0	0	0	0	0	1	2	2	1	2	2	2	2	2	1	1	1	2	2	2	1	1	2
after 24 hours		1	1	2	2	1	2	0	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
after 48 hours		1	1	1	2	1	2	1	1	1	1	1	1	2	2	2	2	2	2	2	2	1	1	2	2	2	2	2	2	2	2
after 72 hours		0	1	1	2	1	2	0	0	0	0	1	1	1	2	2	2	2	2	2	1	1	1	2	2	2	2	2	2	2	2
mean scores 24-72h		1.3						0.7						1.9						1.4											
after 4 days		0	1	1	1	1	1	0	0	0	0	0	0	1	1	1	2	2	2	0	1	1	1	1	1	2	0	0	0	0	0
after 7 days		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	-0	0
after 8 days		-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-	-	0	-	-	-	-	0	-

Conclusion by the Notifiers

Glyphosate acid is a moderate irritant (class 5 on a 1-8 scale) to the rabbit eye.

Comment by RMS:

The study is considered acceptable. The test substance revealed irritating properties to rabbit eyes based on Draize scheme as seen in Table B.6.2-31 [Notifiers conclusion based on modified form of Kay and Chalandra system as mentioned above].

Reference: IIA, 5.2.5/12
Report: [REDACTED] 2007, Glyphosate Technical Material: Primary Eye Irritation Study In Rabbits
 [REDACTED]
 Data owner: Syngenta
 Report No.: B02788
 Date: 2007-03-26, not published
 ASB2012-11437
Guidelines: OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2004)
Deviations: None.
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test Material: Glyphosate Technical Material
 Description: Technical, white powder
 Lot/Batch number: 0507
 Purity: 96.1 % w/w Glyphosate acid
 CAS#: [REDACTED]
 Stability of test compound: Stable under storage conditions of room temperature (range of 20 °C ± 5 °C), protected from light and humidity.
 Vehicle and/or positive control: The test substance was undiluted.
 Test Animals:
 Species: Rabbit
 Strain: New Zealand White (SPF)
 Age/weight at dosing: Male: 11-12 weeks / 2640 g; Females: 14-16 weeks / 2990 and 3001 g
 Source: [REDACTED]
 Housing: Individually in stainless steel cages equipped with feed hoppers and drinking water bowls.
 Acclimatisation period: 5/6 days
 Diet: Pelleted standard Provimi Kliba 3418 rabbit maintenance diet *ad libitum*.
 Water: Community tap water *ad libitum*
 Environmental conditions: Temperature: 17-23 °C
 Humidity: 30-70 %
 Air changes: 10-15 per hour.
 Photoperiod: 12 hours light, 12 hours dark.

Study design and methods

In-life dates: Start: 27 December 2006 End: 4 January 2007

Animal assignment and treatment: On the day of treatment, 0.1 g of Glyphosate Technical Material was placed into the conjunctival sac of the left eye of each animal after gently

pulling the lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of the test substance. The right eye remained untreated and acted as the reference control. The treated eyes were not rinsed after instillation of the test substance.

As it was suspected that the test substance might produce irritancy, a single female was treated first. As neither a corrosive effect nor a severe irritant effect was observed after 1- and 24-hour examinations, the test was completed using the two remaining animals.

The ocular reaction (ie. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, at approximately 1, 24, 48 and 72 hours, as well as 7 days after instillation. Additionally, ocular discharge, reddening of the sclerae and staining of conjunctivae, sclerae and cornea by the test substance was assessed according to the scheme presented in the guideline.

The animals were observed daily throughout the study for viability, mortality and clinical signs. Bodyweights were measured at the start of acclimatisation, on the day of treatment and at termination of the observation period.

Results and discussion

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. No abnormal findings were observed in the cornea or iris of any animal at any of the measurement intervals.

Moderate reddening of the conjunctivae was noted in all animals at the 1-hour reading and persisted in one animal as slight until the 48-hour reading and in two animals as moderate to slight until 72 hours after treatment. Slight to obvious swelling (chemosis) was observed in all three animals at the 1-hour reading and persisted as slight in one animal until the 48-hour reading and in one animal as moderate until 24 hours after instillation. Slight to moderate reddening of the sclerae was noted in all animals at the 1- and 24 hour reading and persisted as slight reddening until the 48-hour reading. Slight to moderate ocular discharge was seen in all animals at the 1-hour reading and persisted as slight to moderate discharge in two animals at the 24-hour reading. No abnormal findings were observed in the treated eye of any animal 7 days after treatment, the end of the observation period for all animals. No staining of the treated eyes produced by the test substance was observed and no corrosion of the cornea was observed at any of the reading times.

Table B.6.2-32: Eye irritation scores of Glyphosate Technical according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004

Time	Animal number	Cornea			Iris			Conjunctiva					
		31M	32F	33F	31M	32F	33F	Redness			Chemosis		
								31M	32F	33F	31M	32F	33F
after 1 hour		0 rd	0 rd	0 rd	0	0	0	2	2	2	1	2	2
after 24 hours		0 ^r	0 rd	0 rd	0	0	0	1	2	2	0	1	2
after 48 hours		0 ^r	0 ^r	0 ^r	0	0	0	1	2	2	0	0	1
after 72 hours		0	0	0	0	0	0	0	1	1	0	0	0
mean scores 24-72h		0	0	0	0	0	0	0.67	1.67	1.67	0	0.33	1
After 7 days		0	0	0	0	0	0	0	0	0	0	0	0

M = male, F = female, d = discharge, r = reddening of the sclerae.

Conclusion by the Notifiers

The instillation of Glyphosate Technical Material into the eye resulted in mild, early-onset and transient ocular changes. These effects were reversible and were no longer evident 7 days

after treatment. Thus, the test item did not induce significant or irreversible damage to the rabbit eye.

Comment by RMS:

The study is considered acceptable and the test substance is irritating to eyes.

Reference: IIA, 5.2.5/13

Report: [REDACTED] 2011, Glyphosate technical: Acute eye irritation study in rabbits

Data owner: Syngenta

Report No.: 10/218-005N

Date: 2011-05-13, not published

ASB2012-11438

Guidelines: OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2008)

Deviations: None.

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test Material: Glyphosate technical

Description: Technical, dry white powder

Lot/Batch number: 569753(BX20070911)

Purity: 96.3 % w/w Glyphosate technical

CAS#: Not reported

Stability of test compound: Stable under storage conditions (room temperature range <30 °C); recertification date end August 2011

Vehicle and/or positive control: None

Test Animals:

Species: Rabbit

Strain: New Zealand White

Age/weight at dosing: Approximately 12 weeks / 3035 g

Source: [REDACTED]

Housing: Individually in metal cage

Acclimatisation period: 13 days

Diet: Purina Base – Lap gr. diet (AgribrandsEurope Hungary PLC, H-5300 Karcag, Madarasi út, Hungary) *ad libitum*

Water: Municipal tap water *ad libitum*

Environmental conditions: Temperature: 20±3 °C

Humidity: 24-64 %

Air changes: 15-20/hour

Photoperiod: 12 hours light/12 hours dark

Study design and methods

In-life dates: Start: 21 December 2011 End: 22 December 2011

Animal assignment and treatment: The primary eye irritation potential of Glyphosate Technical (96.3 % w/w glyphosate technical) was investigated according to OECD test guideline no. 405. Approximately 1 hour before the start of the test, the eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect using a hand-held slit-lamp. The animal used in the study was free of ocular damage. Initially, a single rabbit was treated.

An amount of 0.1 g of the test material was placed into the conjunctival sac of the left eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about 1 second immediately after treatment, to prevent loss of the test material, and then released. The right eye remained untreated and was used for control purposes.

Immediately after administration of the test material, an assessment of the initial pain reaction was made according to 0-5 scale. Following review of the ocular responses produced in the first treated animal, no further animals were treated. The treated eyes were not rinsed after instillation.

The ocular reaction (i.e. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed approximately 1 and 24 hours following treatment, according to the numerical evaluation described by Draize.

The treated eye was further examined using 2 % fluorescein solution before treatment and then 24 hours after treatment.

Additionally, any other signs of eye irritation were recorded.

Results and discussion

No clinical signs of systemic toxicity were observed in the animal during the study and no mortality occurred. The body weight was considered to be within the normal range of variability.

An initial pain reaction score of 3 (on a 0-5 scale) was recorded.

Conjunctival redness, chemosis and conjunctival discharge, as well as corneal opacity, were observed in the rabbit 1 and 24 hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, a few black points on the conjunctiva and dry surface of the eye were noted one hour after the treatment. Fluorescein staining was positive at the 24 hours observation.

Based on the symptoms, no further animals were dosed and the study was terminated after the 24 hour observation (Regulation (EC) No 440/2008).

Table B.6.2-33: Eye irritation scores of Glyphosate Technical according to the Draize scheme

Time	Cornea	Iris	Conjunctiva		
			Redness	Chemosis	Discharge
after 1 hour	2	0	2	3	3
after 24 hours	3	1	3	4	3

Conclusion by the Notifiers:

Under the conditions of this study, Glyphosate Technical is classified as corrosive to the eye.

Comment by RMS:

The study is considered acceptable. The conclusion by the Notifiers is supported.

B.6.2.6 Skin sensitisation

For the previous evaluation a variety of studies on glyphosate acid and two studies on glyphosate salts (IPA) regarding skin sensitising were available. For the current re-evaluation, several additional studies for skin sensitising effects on glyphosate acid were provided: 8 Magnusson-Kligman Tests and 3 Buehler Tests in guinea pigs and two Local lymph node assays in mice. No further studies on glyphosate salts were provided.

Table B.6.2-34: Summary of skin sensitisation studies with glyphosate acid

Reference (Data owner)		Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Results
Studies from the 2001 evaluation	Annex B-5.2.7.1, Glyphosate Monograph [REDACTED], 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	MKT	Not sensitising
	Annex B-5.2.7.1, Glyphosate Monograph [REDACTED] 1993 (Luxan) TOX9650652	Guinea pig English	48 (both sexes)	≥ 95	Intradermal induction: 5% in propylene glycol; topical: 50% in petrolatum	MKT	Not sensitising
	Annex B-5.2.7.1, Glyphosate Monograph [REDACTED] 1991 (AGC) 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	MKT	Not sensitising
	Annex B-5.2.7.1, Glyphosate Monograph [REDACTED] 1989e (CHE) TOX9552343	Guinea pig Dunkin Hartley	46 ♀	98.6	induction: 10% in water; challenge: 25% in water	MKT	Not sensitising
Studies not reviewed in the 2001 evaluation	IIA 5.2.6/01 [REDACTED], 2007 (NUF) 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	MKT	Not sensitising
	IIA 5.2.6/02 [REDACTED] 2010 (HAG) ASB2012-11440	Guinea pig, Dunkin Hartley	15 ♀ (+ 20 for positive control)	96.4	Intradermal induction: 0.01% in water; topical induction: 50%; challenge: 25%	MKT	Not sensitising

Reference (Data owner)		Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Results
	IIA 5.2.6/03 [REDACTED] 1995 (ALS) 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	MKT	Not sensitising
	IIA 5.2.6/04 [REDACTED] 2009 (EXC) ASB2012- 11442	Guinea pig	15 ♂	96.66	Intradermal induction: 10% (w/w) in purified water; topical induction: 50% (w/w) in purified water; challenge: 15% (w/w) in purified water	MKT	Not sensitising
	IIA 5.2.6/05 [REDACTED] 2009 (HAG) ASB2012- 11443	Guinea pig	15 ♂ (+ 20 for positive control)	98.8	Intradermal induction: 0.01% in water, topical induction: 50%; challenge: 50%	MKT	Not sensitising
Studies not reviewed in the 2001 evaluation	IIA 5.2.6/06 [REDACTED], 2010 (HAG) ASB2012- 11444	Guinea pig	15 ♂ (+ 20 for positive control)	97.3	Intradermal induction: 0.5% in water; topical induction: 50%; challenge: 25%	MKT	Not sensitising
	IIA 5.2.6/07 [REDACTED] 2009 (HAG) ASB2012- 11445	Guinea pig	15 ♂ 15 ♀	96.4	400 mg moistened induction and challenge	Buehler Test	Not sensitising
	IIA 5.2.6/08 [REDACTED] 2005 (HAG) ASB2012- 11446	Guinea pig	30 ♀	97.23	70 % in water induction and challenge	Buehler Test	Not sensitising
	IIA 5.2.6/09 [REDACTED] 2008 (HAG) ASB2012- 11447	Guinea pig	30 ♂	98.05	50 % inductions and challenge	Buehler Test	Not sensitising
Studies not reviewed in the 2001 evaluation	IIA 5.2.6/10 [REDACTED] 2006 (NUF) ASB2012- 11448	Guinea pig	20 ♀/test 10 ♀/control	95.7	Intradermal induction: 0.195% (w/v) in isotonic saline; topical induction: 60% (w/v) in water; challenge: 60% (w/v) & 30% (w/v) in water	MKT	Not sensitising

Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Results
IIA 5.2.6/11 [REDACTED], 1996 (SYN) 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	MKT	Not sensitising
IIA 5.2.6/12 [REDACTED], 2007 (SYN) ASB2012- 11449	Mouse CBA	4 ♀/group	96.1	Glyphosate acid dose levels: 0, 10, 25, 45 (% w/v) Hexylcinnamaldehyd e positive control demonstrated sensitivity of study	LLNA	Not sensitising
IIA 5.2.6/13 [REDACTED], 2011 (SYN) ASB2012- 11450	Mouse CBA	4 ♀/group	96.3	Glyphosate acid dose levels: 0, 10, 25, 50 (% w/v) Hexylcinnamaldehyd e positive control demonstrated sensitivity of study	LLNA	Not sensitising

MKT = Magnusson Kligman Maximisation Test

LLNA = Local Lymph Node Assay

Table B.6.2-35: Summary of skin sensitisation studies with glyphosate salts (provided by the Notifiers)

Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Result
Studies from the 2001 evaluation Annex B.5.2.7.2, Glyphosate Monograph [REDACTED] 1994 (MON / CHE) TOX9552345	Guinea pig, Dunkin Hartley	38 ♀	IPA 62.2 %	Induction: 25 % w/v (injection) and 100 % (topical); challenge: 100 %	MKT	Not sensitizing
Annex B.5.2.7.2, Glyphosate Monograph [REDACTED] 1984* (MON) Z35238	Guinea pig	16	IPA 53.8 %	Induction and challenge: 100 %	Modified Buehler Test	Not sensitising

* Study was considered supplementary data in the 2001 EU glyphosate evaluation

MKT = Magnusson Kligman Maximisation Test

Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.

For details regarding studies reviewed during the 2001 EU evaluation please refer to the Monograph.

Reference: IIA, 5.2.6/01

Report: [REDACTED] 2007, Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test

[REDACTED]
[REDACTED] Study No.: B02316

Data owner: Nufarm

Date: 2007-03-06, unpublished

ASB2012-11439

Guidelines: OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), JMAFF guideline 2-1-6 (2005)

Deviations: No

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material: Glyphosate technical

Identification: NUP 05068

Description: Solid

Lot/Batch #: 200609062

Purity: 95.1 %

Stability of test compound: Stable under storage conditions ($20 \pm 5^{\circ}\text{C}$), light protected;
Expiry date: 2008-09-14

Vehicle and/or positive control: Polyethylene glycol 300 (PEG 300)

Test animals:

Species: Guinea pig

Strain: Albino Dunkin Hartley, CRL:(HA)BR, SPF

Source: [REDACTED]

Age: 6 - 6 weeks

Sex: female

Weight at dosing: Pre-tests: 362 – 372 g; main test: 337 – 381 g

Acclimation period: Main test: at least 10 days

Diet/Food: Pelleted standard Provimi Kliba 3418 guinea pig breeding / maintenance diet (Provimi Kliba AG, CH-Kaiseraugust), *ad libitum*

Water: Tap water, *ad libitum*

Housing: Individually in Makrolon type 4 cages with standard softwood bedding

Environmental conditions: Temperature: $22 \pm 3^{\circ}\text{C}$
Humidity: 30 - 70 %
Air changes: 10 - 15/hour
12 hours light/dark cycle

Study design and methods:

In life dates: 2007-01-10 to 2007-02-15

Animal assignment and treatment:

Glyphosate technical (NUP 05068) was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Female Dunkin Hartley guinea pigs, young adults with body weights ranging from 337 to 381 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with three animals. The main study was performed in 20 test animals and 10 control animals.

The induction phase consisted of an intradermal injection at day 1 and an epidermal application on day 8. On day 1 the test substance was injected (0.1 mL/site) into the clipped dorsal skin from the scapular region at a concentration of 3 % either in PEG 300 or in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline. On day 8 the test substance was topically applied at a concentration of 50 % to the clipped and shaved skin of the scapular area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 24 and 48 hours after removal of the bandage.

The challenge was conducted on day 22 by an occlusive patch containing 0.2 mL of the test material at a concentration of 25 % in PEG 300 that was applied to the clipped and shaved left flank of each animal for 24 h. The clipped and shaved right flank of each animal was treated in the same way with the vehicle only (PEG 300). 24 and 48 hours after removal of the dressing skin reactions were scored according to the Magnusson and Kligman grading scale.

Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period.

A positive control (reliability check) with a known sensitizer was not included in this study. However, a separate study was performed from June to August 2006 in the laboratory. The positive controls with alpha-hexylcinnamaldehyde (3 % in PEG 300) showed that the chosen guinea pig strain was able to detect sensitizing compounds under the laboratory conditions chosen.

Evaluation criteria for classification as a potential skin sensitizer:

At the 24-hour and/or 48-hour reading, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

Results and discussion

Mortality: No deaths occurred.

Clinical observations: No signs of systemic toxicity were observed.

Body weight: All animals showed the expected gain in body weight with the exception of one of the pre-test animals that did not gain body weight between the day of epidermal application and day of sacrifice one week later.

Necropsy: No necropsy was performed.

Skin reactions: No skin reactions were observed 24 or 48 h after the challenge treatment with glyphosate technical (NUP 05068) in the control or test group.

Conclusion by the Notifiers:

Based on the EU classification criteria, glyphosate technical (NUP 05068) is not to be classified for skin sensitization. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (NUP 05068) is also not classified for skin sensitization.

Comment by RMS:

The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference: IIA, 5.2.6/02

Report: [REDACTED] 2010, Examination Of Glyphosate TC In The Skin Sensitisation Test In Guinea Pigs According To Magnusson And Kligman (Maximisation Test)

[REDACTED]
Data owner: Helm AG

Report No.: 24879

Date: 2010-01-22, unpublished

ASB2012-11440

Guidelines: OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), OPPTS 870.2600 (1998)

Deviations: None

GLP: yes

Acceptability: *Please see comment by RMS*

Materials and methods

Test material:

Identification: Glyphosate TC

Description: White powder

Lot/Batch #: 2009051501

Purity: 96.4 %

Stability of test compound: At room temperature in the dark stable until May 15, 2011.

Vehicle and/or positive control: Purified water

Test animals:

Species: Guinea pig

Strain: Dunkin Hartley

Source: [REDACTED]

Age: 32 days

Sex: Female

Weight at dosing: 312 - 355 g; positive control group: 249 - 317 g

Acclimation period: At least 5 days.

Diet/Food: ssniff Ms-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum*

Water: Tap water, *ad libitum*

Housing: In pairs in Makrolon cages (MZK 80/25) with granulated textured wood bedding

Environmental conditions: Temperature: 22 ± 3 °C

Humidity: 55 ± 15 %

Air changes: no data

12 hours light/dark cycle

Study design and methods:

In life dates: 2009-10-15 to 2009-11-28

Animal assignment and treatment:

Glyphosate TC was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Female Dunkin Hartley guinea pigs, young adults with body weights ranging from 312 to 355 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals. The main study was performed in 10 test animals, 5 control animals and 20 positive control animals.

The induction phase consisted of an intradermal injection at Day 0 and an epidermal application on Day 7. On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin of the shoulder region at a concentration of 0.01% in *aqua ad iniectabilia*, together with injections of Freund's Complete Adjuvant in physiological saline, or test item in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.

On Day 6 the skin was shaved and coated with 0.5 mL sodium laurylsulfate 10 % in vaseline in order to induce a local irritation. On Day 7 the test substance was topically applied at a concentration of 50 % to the clipped and shaved skin of the shoulder region using the patch technique. The patch was left occluded in place for 48 hours.

The challenge was conducted on Day 21 by an occlusive patch at a concentration of 25 % in *aqua ad iniectabilia* which was applied to the clipped and shaved left flank of each animal for 24 h. The clipped and shaved right flank of each animal was treated in the same way with the vehicle alone. 24 and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale.

The animals of the positive control group were treated with a 2 % benzocaine solution intracutaneously in the induction phase and with a 5 % solution topically in the induction phase and at challenge.

Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period.

Evaluation criteria for classification as a potential skin sensitizer:

At the 24-hour and/or 48-hour readings, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

Results and discussion

Mortality: No deaths occurred.

Clinical observations: No signs of systemic toxicity were observed.

Body weight: All animals showed the expected gain in body weight.

Necropsy: No necropsy was performed.

Skin reactions: No skin reactions were observed 24 or 48 h after the challenge treatment with glyphosate TC in the control or test group.

Animals treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Conclusion by the Notifiers

Based on the study results and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, glyphosate TC is not to be classified for skin sensitization.

Comment by RMS:

The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference: IIA, 5.2.6/03

Report: [REDACTED] 1995, HR-001: Dermal sensitisation study in Guinea pigs.

Study No.: IET 95-0036

Data owner: Arysta LifeScience

Date: 1995-06-28, Unpublished

ASB2012-11441

Guidelines: U.S. EPA FIFRA Guideline Subdivision F

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material: Glyphosate technical

Identification: HR-001

Description: White crystal

Lot/Batch #: T-941209

Purity: 97.56 %

Stability of test compound: Not mentioned in the report

Vehicle and/or positive control: Polyethylene glycol 300 (PEG 300)

Test animals:

Species: Guinea pig

Strain: Hartley, Crj:Hartley

Source: [REDACTED]

Age: 6 weeks

Sex: female

Weight at dosing: 332 – 423 g at the time of intradermal injection

Acclimation period: 1 week

Diet/Food: Pellet diet GC4 (Oriental Yeast Co., Ltd.), *ad libitum*

Water: Filtered and sterilised water, *ad libitum*

Housing: Aluminium cage with wire-mesh floor

Environmental conditions: Temperature: 23.9 °C

Humidity: 51.8 – 56.3 %

Air changes: 15/hour

12 hours light/dark cycle

Study design and methods:

In life dates: 2007-01-10 to 2007-02-15

Animal assignment and treatment:

The test was carried out according to the maximization method of Magnusson and Kligman.

20 female specific pathogen free Hartley guinea pigs (Crj:Hartley) were exposed to concentrations of 5 %, 25 % and 25 % glyphosate technical for intradermal induction (3 pairs of injection), topical induction (applied with an occlusive dressing) and challenge (applied with an occlusive dressing) respectively. These doses selected for both induction and challenge application in the main study were based on the results of range-finding studies. DCNB (2,4-dichlorobenzene) was used (in a 10 female group) as the positive control substance at concentrations of 0.1 %, 1 % and 0.5 % for intradermal induction, topical induction and challenge, respectively. Groups of 10 and 20 animals were used for the negative control group for DCNB (treated with DCNB at the challenge but not at the induction) and negative control group for technical glyphosate (treated with test substance at the challenge but not at the induction), respectively. Skin reaction to the challenge was observed 24 and 48 hours after removal of the patch and dermal sensitisation rates were calculated. Body weights were measured at the first induction and 48 hours after the removal of the patch.

Results and discussion

Mortality: One animal died in the DNCB treatment group. At necropsy of the dead animal, consolidation of lung and hydrothorax were noted. These findings were associated with the hindrance of circulation and the respiratory abnormality, which led to the death. The remaining animals in this group did not show any abnormality in the health condition and the skin reactions were clearly observed.

Clinical observations: No signs of systemic toxicity were observed.

Body weight: No abnormal body weight changes were noted in any animal of the four groups.

Necropsy: No necropsy was performed.

Skin reactions: No oedema or erythema were observed in test animals following challenge with 25 % technical glyphosate. The rate of sensitization in the test substance treatment group was therefore 0 %.

On the other hand, the rate of sensitization in the DCNB treatment group was 100 %, which was considered to sufficiently assure the reliability of this study.

Conclusion by the Notifiers

Based on the EU classification criteria, glyphosate technical (HR-001) is not to be classified for skin sensitization. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (HR-001) is also not classified for skin sensitization.

Comment by RMS:

The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference: IIA, 5.2.6/04

Report: [REDACTED] 2009, Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test

[REDACTED] Data owner: Excel

Report No.: C22908

Date: 2009-05-15, not published

ASB2012-11442

Guidelines:

OECD 406 (1992)

Commission Regulation (EC) No 440/2008 (2008); method B.6

Deviations: None
GLP: yes
Acceptability: Please see comment by RMS

Materials and methods

Test material:
 Identification: Glyphosate Technical
 Description: Solid
 Lot/Batch #: GI-1045
 Purity: 96.66 %
 Stability of test compound: Expiry date: July 2010
 Test item dilution: Stable in purified water for 2 days.
 Vehicle and/or positive control: Purified water
 Test animals:
 Species: Guinea pig
 Strain: Albino Dunkin Hartley, CRL:(AH)BR, SPF
 Source: [REDACTED]
 Age: 4 – 6 weeks (at pre-test / at beginning of acclimatization period)
 Sex: Male
 Weight at dosing: 348 – 358 g (at pre-test)
 335 – 365 g (at beginning of acclimatization period)
 Acclimation period: Approx. 2 weeks (for main study)
 Pellet standard Provimi Kliba 3418 guinea pig breeding / maintenance diet batch nos. 55/08 and 72/08, containing Vitamin C (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland), *ad libitum*.
 Diet/Food:
 Water: Tap water, *ad libitum*
 Housing: Individually in Makrolon type-4 cages with standard softwood bedding ("Lignocel", Schill AG, 4132 MuttENZ / Switzerland)
 Environmental conditions: Temperature: 22 ± 3 °C
 Humidity: 30 – 70 %
 Air changes: 10 - 15/hour
 12-hour light/dark cycle

Study design and methods:

In life dates: 2009-01-14 to 2009-02-27

Animal assignment and treatment:

Glyphosate Technical was tested for its sensitizing effect on the skin of guinea pig in the Maximization-Test according to Magnusson-Kligman. Fifteen (10 test and 5 control) male Albino Dunkin Hartley CRL:(AH)BR, SPF guinea pigs, 4-6 weeks of age, and 335-365 g of weight were employed for this study.

The concentrations of test substance for the main test were selected based on the results of a pre-test (during the acclimatization period of the main animals).

The intradermal induction of sensitisation in the test group was performed in the nuchal region with a 10 % dilution of the test item in purified water and in an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. The epidermal induction of sensitization was conducted for 48 hours under occlusion with the test item at 50 % in purified water one week after the intradermal induction. The animals of the control group were intradermally induced with purified water and FCA/physiological saline and epidermally induced with purified water under occlusion.

Two weeks after epidermal induction the control and test animals were challenged by epidermal application of the test item at 15 % in purified water and purified water alone under occlusive dressing.

Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing (according to the criteria laid down in test guidelines).

A positive control (reliability check) with a known sensitizer was not included in this study. However, a separate study was performed in the laboratory. The positive controls with α -hexylcinnamaldehyde at 3 % in PEG 300 showed that the chosen guinea pig strain was able to detect sensitising compounds under the laboratory conditions chosen.

Body weights were determined at delivery/acclimatization start, at the end of the pretest, at test day 1 (day of treatment), and at the termination of the study. Mortality was checked daily.

Results and discussion

Mortality: There were no treatment related deaths during the course of the study, hence no necropsies were performed.

One pre-test animal was found in bad conditions before the start of pre-test and during the acclimatization period of the main test animals. This animal was sacrificed for ethical reasons and replaced by a new animal.

Clinical observations: No signs of systemic toxicity were observed in the animals.

Body weight: The body weight of the animals was within the range commonly recorded for animals of this strain and age.

One animal lost visible amount of body weight (31 %) before the start of the intradermal pre-test. It was killed for ethical reasons and replaced by another animal.

Necropsy: No necropsies were performed.

Skin reactions: Skin Effects in the Intradermal Induction (Test Day 1)

The expected and common findings were observed in the control and test group after the different applications using FCA intradermally. These findings consisted of erythema, oedema, necrotizing dermatitis, encrustation and exfoliation of encrustation.

Skin Effects in the Epidermal Induction (Test Day 8)

Control group – No erythematous or oedematous reaction was observed in the animals treated with purified water only.

Test group – Discrete/patchy erythema was observed in eight out of ten test animals at the 24-hour observation and persisted in seven animals up to the 48-hour reading after treatment with the test item at 50 % in purified water.

Skin Effects in the Challenge Procedure

Control group and Test group – No positive/skin reactions were observed in the animals when treated with either purified water only or when treated with the test item at 15 % in purified water.

Conclusion by the Notifiers

Based on the above mentioned findings in the Magnusson & Kligman Test in guinea pigs and in accordance to Commission Directive 2001/59/EC, Glyphosate Technical does not have to be classified and labelled as a skin sensitizer.

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate technical is not to be classified for skin sensitization.

Comment by RMS:

The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference: IIA, 5.2.6/05

Report: [REDACTED] 2009, Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test)

[REDACTED]
Data owner: Helm AG
Report No.: LPT 23915
Date: 2009-05-19, unpublished
ASB2012-11443

Guidelines: OECD 406
US EPA OPPTS 870.2600

Deviations: Personnel change of the head of the Quality Assurance Unit: until January 31, 2009: Dipl. Biol. S. Steuer
as of February 1, 2009: Dr. med. vet. habil. K. R. Sultan.
This minor deviation did not have any effect on the validity and integrity of the scientific results obtained in this.

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	Aqua ad iniectabilia/ Benzocaine
Test animals:	
Species:	Guinea pig
Strain:	Dunkin-Hartley

Source:



Age: 32 days

Sex: Male

Weight at dosing: 313 – 358 g
(excluding positive control group)
Positive control group: 271 - 331 g

Acclimation period: At least 5 days

Diet/Food: Commercial diet, ssniffB MS-H V2233 (ssniff Spezialdiäten GmbH) served as food. The food was offered *ad libitum*.

Water: Tap water, *ad libitum*

Housing: The animals were kept in pairs in MAKROLON cages (MZK 80/25). Granulated textured wood (Granulat A2, J. BRANDENBURG, 49424 Goldenstedt, Germany) was used as bedding material in the cages. The cages were changed and cleaned twice a week.

Environmental conditions: Temperature: $22 \pm 3^{\circ}\text{C}$
Humidity: $55 \pm 15\%$
12-hour light/dark cycle

Study design and methods:

In life dates: 2009-02-04 to 2009-03-28

Animal assignment and treatment:

The purpose of this study was to determine the potential of Glyphosate TC to provoke skin sensitisation reactions in guinea pigs. Possible sensitising properties of the test item were evaluated by administration of the test item to the shoulder region, first by intracutaneous application (stage 1) and 7 days later by topical administration (stage 2, exposure time: 48 hours). In a challenge test (stage 3) the test item was again applied topically but to the flank region (exposure time: 24 hours). This area was then examined for reactions which might indicate sensitising properties of the test item.

Induction: The skin reaction results of the first induction exposure were evaluated at 24 and 48 hours, of the second induction at 48 and 72 hours after beginning of exposure.

Challenge:

Days 23 and 24:

21 hours after removing the filter paper the challenge area was cleaned and cleared of hair if necessary three hours later (at 48 hours from the start of challenge application) the skin reaction was observed and recorded. 24 hours after this observation a second observation (72 hours) was made and recorded.

Body weight: Body weight gain was unaffected by the administration of the test substance.

The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Report: [REDACTED] 2010, Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test)

Data owner: Helm AG
Report No.: LPT 24607
Date: 2010-04-19, unpublished
ASB2012-11444

Deviations: The following minor deviations were noted:
The batch no. of the sodium lauryl sulfate was 081 K0078 and not 121 H0370 as stated in the Study Plan.
Freund's complete adjuvant was manufactured by SIGMA-ALDRICH Chemie GmbH, 82024 Taufkirchen, Germany, and not by DIFCO Laboratories Detroit, Michigan, USA, as stated in the Study Plan.
These minor deviations from the Study Plan did not affect the validity and scientific results of the study.

GLP: yes
Acceptability: Please see comment by RMS

Materials and methods


Test material:	Glyphosate
Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %
Stability of test compound:	No data given in the report.

Vehicle and/
or positive control: *Aqua ad iniectabilia*
Benzocaine

Test animals:

Species: Guinea pig

Strain: Dunkin-Hartley

Source: 

Age: 32 days

Sex: Male

Weight at dosing: 299 - 364 g
(excluding positive control group)
Positive control group: 319 - 346 g

Acclimation period: At least 5 days

Diet/Food: Commercial diet, ssniffB MS-H V2233 (ssniff
Spezialdiäten GmbH) served as food. The food was offered
ad libitum

Water: Tap water, *ad libitum*

Housing: The animals were kept in pairs in MAKROLON cages
(MZK 80/25). Granulated textured wood (Granulat A2, J.
BRANDENBURG, 49424 Goldenstedt, Germany) was
used as bedding material in the cages. The cages were
changed and cleaned twice a week.

Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 55 ± 15 %
12-hour light/dark cycle

Study design and methods:

In life dates: 2009-10-26 to 2010-01-30

Animal assignment and treatment:

The purpose of this study was to determine the potential of Glyphosate TC to provoke skin sensitisation reactions in guinea pigs. Possible sensitising properties of the test item were evaluated by administration of the test item to the shoulder region, first by intracutaneous application (stage 1) and 7 days later by topical administration (stage 2, exposure time: 48 hours). In a challenge test (stage 3) the test item was again applied topically but to the flank region (exposure time: 24 hours). This area was then examined for reactions which might indicate sensitising properties of the test item.

Induction: The skin reaction results of the first induction exposure were evaluated at 24 and 48 hours, of the second induction at 48 and 72 hours after beginning of exposure.

Challenge: Days 23 and 24: 21 hours after removing the filter paper the challenge area was cleaned and cleared of hair if necessary three hours later (at 48 hours from the start of challenge application) the skin reaction was observed and recorded. 24 hours after this observation a second observation (72 hours) was made and recorded.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Behaviour of the animals remained unchanged. Given the negative response in all treated animals further testing was not considered necessary in order to reduce animal experiments for animal welfare reasons.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Conclusion by the Notifiers

Under the present test conditions glyphosate TC revealed no sensitising properties in guinea pigs in a test model according to Magnusson and Kligman.

Comment by RMS:

The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference:

IIA, 5.2.6/07

Report:

2009, Glyphosate – Skin Sensitization Study in Guinea Pigs.
Buehler Test.

Data owner: Helm AG

Report No.: 12174-08

Date: 2009-03-11, unpublished

ASB2012-11445

Guidelines:

US EPA OPPTS 870.2600

Equivalent to OECD 406.

Deviations:

Humidity was in the range of 25-98% instead of 30-70%. This deviation did not affect the study outcome

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:

Glyphosate

Identification:

Glyphosate Tech Grade Mixed 5-Batch

Description:

White powder

Lot/Batch #:

080704-1 thru 5

Purity:

96.4 %

Stability of test compound:

No data given in the report.

Vehicle and/or positive control:

Deionised water/
alpha-Hexylcinnamaldehyde

Test animals:

Species:

Guinea Pig

Strain:

Hartley-Albino

Source:

Age:

Approx. 4 weeks

Sex:

Males and Females

Weight at dosing: Males: 359-414 g; Females: 341-387 g
 Acclimation period: 5 days
 Diet/Food: PMI Feeds, Inc.™ Guinea Pig Diet #5025; available *ad libitum*
 Water: Tap water, *ad libitum*
 Housing: Individual housing in suspended, wire bottom, stainless steel cages.
 Environmental conditions: Temperature: 22 ± 3 °C
 Humidity: 30 - 70 %
 Air changes: 10 - 12/hour
 12-hour light/dark cycle

Study design and methods:

In life dates: 2008-11-30 to 2009-01-02

Animal assignment and treatment:

Observations for skin reactions at each test site were made approximately 24 hours after each treatment. In addition, observations for skin reactions were made approximately 48 hours after the first induction treatment and 48 hours after the challenge treatment. An average score for each time period was obtained by adding all of the scores for each time period and dividing by the number of test sites scored for that time period. The test substance is considered a sensitizer if the mean irritation scores, the total number of animals with scores, and/or the total number of scores for the virgin test site in the test group after the challenge treatment are appreciably greater than those for the naive challenge group. The average skin reaction score of this study was 0.0.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The test substance, glyphosate, produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitising reaction in guinea pigs.

Conclusion by the Notifiers

The test substance, glyphosate, produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitizing reaction in guinea pigs and is therefore not sensitising.

Comment by RMS:

The study is considered acceptable and revealed no evidence for skin sensitisation potential of the test substance.

Reference:

IIA, 5.2.6/08

Report:

2005, Glyphosate acid technical – Dermal Sensitization in Guinea Pigs (Buehler Method)

Data owner: Helm AG

Report No.: 15279

Date: 2005-04-04, unpublished

ASB2012-11446

Guidelines: US EPA OPPTS 870.2600
OECD 406.
Deviations: No deviations from the Study Plan
GLP: yes
Acceptability: *Please see comment by RMS*

Materials and methods

Test material: Glyphosate
Identification: Glyphosate Acid Technical
Description: White crystalline powder
Lot/Batch #: 040205
Purity: 97.23 %
Stability of test compound: No data given in the report.
Vehicle and/or positive control: Distilled water/
alpha-Hexylcinnamaldehyde Technical
Test animals:
Species: Guinea pig
Strain: Hartley albino
Source: XXXXXXXXXX
Age: Young adult
Sex: Male and Female
Weight at dosing: 327-391 g
Acclimation period: 5 or 38 days
Diet/Food: Pelleted Purina Guinea Pig Chow #5025
Water: Tap water, *ad libitum*
Housing: The animals were group housed in suspended stainless steel caging with mesh floors or plastic perforated bottom caging.
Environmental conditions: Temperature: 18-22 °C
12-hour light/dark cycle

Study design and methods:

In life dates: 2004-05-03 to 2004-06-03

Animal assignment and treatment:

In order to evaluate the sensitisation response at challenge, two indices were used; one for incidence and one for severity in the test and vehicle control animals. The incidence index was the ratio of animals with erythema scores greater than 0.5 per number of animals evaluated and is presented for both the 24 and 48 hour intervals after challenge evaluation intervals as follows: Incidence Index = Number of erythema scores greater than 0.5 / Number of animals evaluated The severity index is the mean erythema score, and is calculated for both the 24 and 48 hour after challenge evaluation intervals according to the following formula: Severity Index = Sum of erythema scores/Number of animals evaluated. The following criteria were used to classify the test substance as a potential contact sensitizer: At the 24-hour and/or 48-hour scoring interval, 15 % or more of the test animals exhibit a positive

response (scores > 0.5) in the absence of similar results in the vehicle control group. The positive reaction at the 24-hour interval must persist to 48 hours in at least one test animal.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Induction Phase:

Test Animals (70 % w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was observed for most test sites during the induction phase.

Test Animals (70 % w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was observed at six of twenty test sites 24 hours following the challenge application. Similar irritation persisted at one affected site through 48 hours.

Conclusion by the Notifiers

Based on these findings and on the evaluation system used, Glyphosate Acid Technical is not considered to be a contact sensitizer.

Comment by RMS:

The study is considered acceptable. Based on the study conditions no evidence for skin sensitisation potential of the test substance was observed.

Reference:

IIA, 5.2.6/09

Report:

██████████ B.S. 2008, Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs, Buehler Test.

██████████
Data owner: Helm AG

Report No.: RF-3996.318.431.07

Date: 2008-09-30, unpublished

ASB2012-11447

Guidelines:

OECD 406.

Deviations:

1. The experimental phase initiation and experimental phase conclusion dates were updated,
 2. The test item was applied using DMSO as vehicle.
- The deviations listed above had no negative impact on the outcome of the study.

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test material:	Glyphosate
Identification:	Glyphosate Technical
Description:	White powder
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.
Vehicle and/or positive control:	DMSO
Test animals:	

Species:	Guinea Pig
Strain:	Hartley
Source:	
Age:	Eight to nine weeks old
Sex:	Male
Weight at dosing:	444 - 556 g
Acclimation period:	7 days
Diet/Food:	Pelleted commercial diet - "Nuvilab Cobaias 6001"
Water:	Tap water, <i>ad libitum</i>
Housing:	88 x55 x 28 cm polypropylene cages with autoclaved wood shavings containing five animals per cage during the experimental phase were used
Environmental conditions:	Temperature: 18 - 23 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods:

In life dates: 2008-06-12 to 2008-07-12

Animal assignment and treatment:

The contact sites to the skin (left flank for inductions and right flank for challenge) at each application day were mechanically and closely clipped free of hair using an electrical razor. Skin was observed for lesions after clipping the fur. According to the Buehler's application method, animals were exposed to cotton lint patches with an approximated 6 cm² surface area. Treatment animals were exposed to patches loaded with 1 mL of 50 % (w/v) test solutions in DMSO (equivalent 0.5 g of the test item) for the induction and challenge applications. Control animals were exposed to patches loaded with 1 mL of vehicle on inductions and loaded with 1 mL of test solution on challenge, therefore being submitted to the same procedures as treatment animals, except on inductions. Since control animals were not exposed to the test item on inductions, a hypersensitive state could not be induced in these animals, which then constituted a negative control in order to allow the differentiation between skin irritation and skin sensitisation at challenge. Patches were held in contact with the skin by an occlusive dressing during an approximated 6-hour exposure period in each application, after which patches were carefully removed from the skin and any residue cleaned up using DMSO. Four applications were carried out, with a seven-day interval between inductions and a fourteen-day interval between third induction and challenge. Animals were clinically examined approximately 30 and 54 hours after each application. Skin reactions were evaluated in agreement with Magnusson & Kligman's grading scale after challenge application.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Neither compound-related clinical signs nor behavioral alterations were observed during inductions. No animal from control group was positive for the test item after challenge application. One animal from treatment group was positive for the test item after challenge application.

Conclusion by the Notifiers

The epidermal application of glyphosate technical using DMSO as vehicle did not cause skin sensitisation in guinea pigs, according to the Buehler Test Method.

Comment by RMS:

Despite the deviations in the study design, no evidence for skin sensitisation potential of the test substance was observed. The study is considered acceptable.

Reference: IIA, 5.2.6/10

Report: [REDACTED] 2006, Glyphosate Technical: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation method
Data owner: Nufarm
Study No.: SMK-PH-05/2018,
Report No.: 2060/009
Date: 2006-01-13
GLP:
Unpublished
ASB2012-11448

Guidelines: § OECD Guidelines for the Testing of Chemicals No. 406 “Skin Sensitisation” (adopted 17 July 1992)
§ Method B6 Acute Toxicity (Skin Sensitisation) of Commission Directive 96/54/EC
§ Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Testing Guidelines for Toxicology Studies, 12 NohSan No. 8147, Guideline No. 2-1-6, revised on 24 November 2004 and 13 Seisan No. 3986 of October 10, 2001

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test material:	
Identification:	Glyphosate Technical
Description:	white powder
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	No data available
Vehicle and/or positive control:	Distilled water
Test animals:	
Species:	Guinea Pigs
Strain:	albino Dunkin Hartley
Source:	[REDACTED]
Age:	Not specified

Sex:	Female
Weight at dosing:	295 to 370 gg
Acclimation period:	5 days
Diet/Food:	Not specified. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.
Water:	Tap water, ad libitum
Housing:	in groups of two or three in makrolon cages furnished with woodflakes
Environmental conditions:	Temperature: 19 to 25 °C Humidity: 30 to 70 % Air changes: not specified 12-hour light/dark cycle

Study design and methods:

In life dates: 05 September 2005 to 13 October 2005

Animal assignment and treatment:

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson & Kligman. A group of thirty guinea pigs was used for the main study, twenty test and ten control. Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response. Induction of the Test Animals: A row of three injections (0.1 ml each) was made on each side of the spine, consisting of a) Freund's Complete Adjuvant plus isotonic sodium chloride in the ratio 1:1 b) a 0.195 % (v/v) formulation of the test material in isotonic sodium chloride c) a 0.195 % (v/v) formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. On Day 6, the scapular region of all test and control animals was shaved and sodium lauryl sulphate (10 % in petroleum jelly) was spread evenly over the area to create local irritation. On Day 7 the same area on the shoulder region used previously for intradermal injections was treated with a topical application of the test material formulation (60 % (w/w) in distilled water) under occlusive dressing for 48 hours. The intradermal induction on the control animals was performed using an identical procedure without the test material. Injection b) was therefore the vehicle alone, injection c) was a 50 % formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted. For the challenge phase, test material formulation at the maximum non-irritant concentration (60 % (w/w) in distilled water) was applied to one side of the shorn flank of each animal under an occlusive dressing. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 30 % (v/v) in distilled water was similarly applied under an occlusive dressing to the opposite skin site on the shorn flank. After 24 hours, the dressing was carefully removed and discarded. The topical challenge sites were cleaned if required. Prior to the 24-hour observation the flanks were clipped to remove regrown hair. Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified. Any other reactions were also recorded.

None of the animals exposed to test material showed any signs of sensitisation after the challenge phase.

Results and discussion

Mortality: One test group animal was found dead on Day 3 and one other test group animal was found dead on Day 5. The cause of death was not determined but was considered not to be treatment related. The absence of these animals was considered not to affect the purpose or integrity of the study.

Clinical observations: The concentration chosen for use in the Intradermal Induction phase of the main test was 0.195 % (v/v) in isotonic sodium chloride solution (maximal non necrotising concentration (MNNC)). The concentration chosen for use in the Topical Induction phase of the main test was 60% (w/w) in distilled water (maximal non irritant concentration (MNIC)). The concentrations chosen for use in the Topical Challenge phase of the main test were 60 % (w/w) (maximal non irritant concentration (MNIC)) and 30 % (v/v) in distilled water (1/2 MNIC).

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The test material produced a 0 % (0/18) sensitisation rate and was classified as a non-sensitiser to guinea pig skin under the conditions of the test. The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations Commission Directive 2001/59/EC. No symbol and risk phrase are required.

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

Comment by RMS:

The study is considered acceptable. Based on the study conditions no evidence for skin sensitisation potential of the test substance was observed.

Reference:

Report:

IIA, 5.2.6/

96, Glyphosate Acid: Skin Sensitisation To The Guinea

Data owner: Syngenta

Report No.: /P/4699

Date: 1996-08-23, not published

TOX2000-1987

Guidelines:

OECD 406 (1992): OPPTS 870.2600 (2003): 96/54/EC B.6 (1996)

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test Material:

Glyphosate acid

Description:

Technical, white solid

Lot/Batch number:

P24

Purity:

As given in report 95.6 % a.i

CAS#:

Not reported

Stability of test compound:	Not reported
Vehicle and/or positive control:	Deionised water
Test Animals:	
Species	Guinea pig
Strain	Albino Cr1 (HA) BR
Age/weight at dosing	Young adult females / 250-317 g
Source	
Housing	Individually in suspended cages in racks suitable for animals of this strain and the weight range expected during the course of the study.
Acclimatisation period	At least 6 days
Diet	RGP), supplied by Labsure, Manea, Cambridgeshire, UK <i>ad libitum</i>
Water	Mains water <i>ad libitum</i>
Environmental conditions	Temperature: 17 ± 2 °C Humidity: 40-70 % Air changes: Approximately 25 changes/hour Photoperiod: 12 hours light, 12 hours dark

Study design and methods

In-life dates: Start: 25 April 1995 End: 19 May 1995

Animal assignment and treatment: In a dermal sensitisation study with glyphosate acid (95.6 % w/w a.i.) young adult, female, Albino Cr1 (HA) BR guinea pigs were tested using the method of Magnusson and Kligman. The study involved the treatment of guinea pigs using two procedures: the potential induction of an immune response and a challenge of that response.

Induction: An area approximately 5 x 5 cm on the scapular region of each animal was clipped free of hair and a row of three injections (0.05-0.1 mL each) was made on each side of the mid-line. The injections were:

- i) Top: Freund's Complete Adjuvant plus deionised water in the ratio 1:1;
- ii) Middle: a 0.1 % w/v preparation of the test substance in deionised water;
- iii) Bottom: a 0.1 % w/v preparation of the test substance in a 1: 1 preparation of Freund's Complete Adjuvant plus deionised water.

Control animals were treated the same as the test animals, except that they were treated with deionised water in place of the test substance.

One day prior to topical induction, the application site was clipped and 0.5 mL of a 10 % w/v preparation of sodium lauryl sulphate in paraffin wax was applied in order to provoke a mild inflammatory response.

One week after intradermal injection, the scapular area was treated with a topical application of the test substance as a 75 % w/v preparation in deionised water. This preparation (0.2-0.3 mL) was applied on filter paper (approximate size 4 cm x 2 cm) which was held in place by a piece of surgical tape. The tape was covered by a strip of adhesive bandage (approximate size 20-30 cm x 5 cm) and secured by a piece of self-adhesive PVC tape. This occlusive dressing was kept in place for approximately 2 days.

Deionised water only was applied to the filter paper for control animals.

The application sites were checked approximately 1 day after removal of the dressings.

Challenge: Two weeks after the topical inductions, an area, approximately 15 cm x 5 cm, on both flanks of all the test and control animals, was clipped free of hair. An occlusive dressing was prepared which consisted of two pieces of filter paper (approximate size 1 cm x 1.5-2.0 cm) stitched to a piece of rubber sheeting (approximate size 12 cm x 5 cm).

A 75 % w/v preparation of the test substance in deionised water (0.05-0.1 mL) was applied to one of the pieces of filter paper and a 30 % w/v preparation in deionised water (0.05-0.1 mL) was applied to the second piece of filter paper. The dressing was placed on the shorn flank of the guinea pig so that the 75 % w/v preparation was on the left and the 30 % w/v preparation was on the right. It was then covered with a strip of adhesive bandage (approximate size 25-40 cm x 7.5 cm) which was secured by a self-adhesive PVC tape.

After approximately 1 day, the dressings were carefully removed. Skin sites were examined approximately 1 and 2 days after removal of the dressings and any erythematous reactions were quantified and recorded, using a four-point scale.

Positive Controls: The sensitising potential of hexylcinnamaldehyde (HCA) was assessed essentially as described above to demonstrate the sensitivity of the strain of animals used and the reliability of the experimental technique. A concentration of 0.3 % w/v HCA in corn oil was used for the intradermal injections and HCA was used undiluted for the topical induction and challenge applications.

Results and discussion

Bodyweights: There were no treatment-related effects on bodyweight during the study.

Induction reactions and duration: Not reported.

Challenge reactions and duration: Following challenge of previously-induced guinea pigs with a 75 % w/v preparation of the glyphosate acid in deionised water, scattered mild redness was seen in three of the twenty test animals and one of the ten control animals. This response is considered to be due to skin irritation following topical challenge. The basis for this conclusion is that an equivalent reaction was seen in one of the ten control animals and the reaction was restricted to the 24 hour clinical observation only, which is characteristic of a mild skin irritation reaction rather than skin sensitisation.

Following challenge of previously-induced guinea pigs with a 30 % w/v preparation of the glyphosate acid in deionised water, no reaction was seen in any of the test or control animals. The net percentage response was calculated to be 0 %.

Positive control: Following challenge of previously induced guinea pigs, scattered mild redness or moderate diffuse redness was observed in 14/20 test animals. Scattered mild redness was seen in two of the ten control animals. The net % response was 50 % and, therefore, HCA was classified as a moderate skin sensitiser which demonstrated the sensitivity of the strain of animals used and the reliability of the experimental technique.

Table B.6.2-36: Number of animals with positive signs following challenge

	Test flank			
	Challenge at 75 %		Challenge at 30 %	
Scored after:	24 hours	48 hours	24 hours	48 hours
Main test – test group	3/20	0/20	0/20	0/20
Main test – negative vehicle control	1/10	0/10	0/10	0/10
	Challenge at 100%			
	24 hours		48 hours	
Positive control – test group	14/20		13/20	
Positive control – vehicle control	2/10		0/10	

Conclusion by the Notifiers:

Glyphosate acid is not a skin sensitiser under the conditions of the test.

Comment by RMS:

Despite reporting deficiencies the study is considered acceptable. Based on the study conditions no evidence of skin sensitising potential was observed.

Reference:

IIA, 5.2.6/12

Report:

2007, Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay In The Mouse).

Data owner: Syngenta

Report No.: R61837/1004

Date: 2007-02-09, not published

ASB2012-11449

Guidelines:

OECD 429 (2002): OPPTS 870.2600 (2003): 2004/73/EC B.42 (2004)

Deviations:

None

GLP:

yes

Acceptability:

Please see comment by RMS

Materials and methods

Test Material:

Glyphosate Technical Material

Description:

Technical, white solid

Lot/Batch number:

0507

Purity:

96.1 %

Stability of test compound:

Stable under storage conditions – ambient temperature in the dark.

Vehicle and/or positive control:

The vehicle for the test substance was dimethyl sulphoxide.
The vehicle for the positive control substance (hexylcinnamaldehyde) was acetone in olive oil.

Test Animals:

Species

Mouse

Strain

CBA/Ca/Ola/Hsd

Age/weight at dosing

8-12 weeks / 16.5-20.8 g

Source

Housing

Maximum 4 per cage, in cages suitable for animals of this strain and weight range.

Acclimatisation period

At least 5 days

Diet

Diet (RM1), supplied by Special Diets Services Limited, Witham, Essex, UK. *ad libitum*

Water

Mains water supplied by an automatic system *ad libitum*

Environmental conditions

Temperature: 22 ± 3 °C

Humidity: 30-70 %

Air changes: A minimum of 15 changes/hour

Photoperiod: Artificial, 12 hours light / 12 hours dark.

Study design and methods

In-life dates: Start: 10th January 2007 End: 16th January 2007

Animal assignment and treatment: A sample of glyphosate technical material was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The assay determines the level of T lymphocyte proliferation in the lymph nodes draining the site of chemical application by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The test substance was applied as 10, 25 or 45 % w/v preparations in dimethyl sulphoxide. Groups of four female CBA/Ca/Ola/Hsd mice were used for this study.

Dose selection rationale: Approximately 25µL of a 10, 25 or 45 % w/v preparation of the test substance was used in this study as 45 % w/v was the limit of solubility.

Treatment preparation and administration: Approximately 25µL of a 10, 25 or 45 % w/v preparation of the test substance in dimethyl sulphoxide was applied, using a variable volume micro-pipette, to the dorsal surface of each ear. A vehicle control group was similarly treated using dimethyl sulphoxide alone. The procedure was repeated daily for 3 consecutive days. Three days after the third application, all the animals were injected, via the tail vein, with approximately 250µL of phosphate buffered saline (PBS) containing 20µCi of a 2.0Ci/mmol specific activity ³H-methyl thymidine. Approximately 5 hours later, the animals were humanely killed by inhalation of halothane vapour followed by cervical dislocation. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in the group, were placed in a container of PBS.

A single cell suspension was prepared by mechanical disaggregation of lymph nodes through a 200-mesh stainless steel gauze. The cell suspensions were then washed three times by centrifugation with approximately 10 mL of PBS. Approximately 3mL of 5 % w/v trichloroacetic acid (TCA) was added and after overnight precipitation at 4 °C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were then resuspended in approximately 1mL of TCA.

The lymph node suspensions were transferred to scintillation vials and 10 mL of scintillant (Optiphase) was added prior to β-scintillation counting using a Packard Tri-Carb 3100TR Liquid Scintillation Counter.

The reliability of the test system was assessed in a positive control study using the same method with a known sensitiser (hexylcinnamaldehyde) applied as 5 %, 10 % or 25 % w/v preparations in acetone in olive oil.

Statistics / Data Evaluation: The results are expressed as a disintegrations per minute (dpm) value per lymph node for each group. The activity of each test group is then divided by the activity of the vehicle control group to give a test:control ratio known as the stimulation index (SI), for each concentration.

The criterion for a positive response is that one or more concentrations of the test substance should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The assay is able to identify those materials that elicit responses in standard guinea pig tests for skin sensitisation. Consequently, a test substance which does not fulfil the above criterion is designated as unlikely to be a skin sensitiser.

Results and discussion

Group Mean Counts per Minute: The application of the test substance at concentrations of 10, 25 and 45 % w/v in dimethyl sulphoxide resulted in an isotope incorporation which was less

than 3-fold at all concentrations. Consequently, the test substance is considered not to be a skin sensitiser under the conditions of the test.

Table B.6.2-37: Radiolabel incorporation into lymph-nodes of mice treated with glyphosate technical material

Concentration of NOA446510 (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio (SI)
0 (vehicle only)	8	3912	489	N/A
10	8	2394	299	0.6
25	8	3292	412	0.8
45	8	4067	508	1.0

N/A = not applicable

In the positive control study, the application of hexylcinnamaldehyde at concentrations of 5 %, 10 % and 25 % w/v in acetone in olive oil (4:1) resulted in a greater than 3-fold increase in isotope incorporation at the 25 % w/v concentration. Therefore, hexylcinnamaldehyde was shown to be a skin sensitiser, confirming the validity of the protocol used for the study.

Table B.6.2-38: Radiolabel incorporation into lymph-nodes of mice treated with the positive control substance (hexylcinnamaldehyde)

Concentration of hexylcinnamaldehyde (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio (SI)
0 (vehicle only)	8	5939	742	N/A
5	8	10111	1264	1.7
10	8	13747	1718	2.3
25	8	38015	4752	6.4

Conclusion by the Notifiers

Glyphosate technical material is considered not to be a skin sensitiser under the conditions of the test.

Comment by RMS:

The study is considered acceptable and no evidence for skin sensitisation potential of the test substance was observed.

Reference: IIA, 5.2.6/13

Report: [REDACTED] 2011, Glyphosate technical: Local lymph node assay in the mouse

[REDACTED]
Data owner: Syngenta

Report No.: 10/218-037E

Date: 2011-04-21, not published

ASB2012-11450

Guidelines: OECD 429 (2010)

Deviations: None

GLP: yes

Acceptability: Please see comment by RMS

Materials and methods

Test Material:	Glyphosate technical
Description:	Technical, dry white powder
Lot/Batch number:	569753 9 (BX20070911)
Purity:	96.3 % w/w glyphosate technical
CAS#:	Not reported
Stability of test compound:	Stable under storage conditions (room temperature range 2-8°C), recertification date end August 2011
Vehicle and/or positive control:	The vehicle for the test substance was propylene glycol
Test Animals:	
Species	Mice
Strain	CBA/J Rj
Age/weight at dosing	9-10 weeks / 20.1-21.6 g
Source	
Housing	Group housed in Type II. polypropylene/polycarbonate cages
Acclimatisation period	13 days
Diet	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany <i>ad libitum</i>
Water	Tap water <i>ad libitum</i>
Environmental conditions	Temperature: 22±3°C Humidity: 30-70% Air changes: 15-20 air changes per hour Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: 20 October 2010 End: 26 October 2010

A sample of glyphosate Technical (96.3 % w/w Glyphosate technical) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay, following dermal exposure when administered topically to young adult, female CBA/J Rj mice.

Animal assignment and treatment:

Dose selection rationale: A Preliminary Irritation/Toxicity Test was performed on CBA/J Rj mice using two doses at test item concentrations of 50 and 25 (w/v)%, respectively. This preliminary experiment was conducted in a similar experimental manner to the main study, but it was terminated on Day 6 without radioactive proliferation assay.

During the Preliminary Irritation/Toxicity Test no mortality, systemic toxicity or local irritation were observed. No treatment related effect on body weights was observed. The observations recorded in this preliminary test suggest that the formulations, the application of the material and the local effects on the animal are acceptable for a valid LLNA.

Based on the results of the preliminary experiments the following dose levels were selected for the main assay: 0 (negative control), 10, 25 and 50 w/v% Glyphosate Technical, and positive control (25 % HCA in PG). Each group comprised four mice.

Treatment and observations: Each animal was topically dosed once a day for 3 consecutive days (Days 1, 2 and 3) on the dorsal surface of each ear with 25 µL of the appropriate formulation, applied using a pipette. There was no treatment on Days 4, 5 and 6.

All animals were observed at least once daily (Days 1-6) for any clinical signs, including local irritation and systemic toxicity. Individual body weights were recorded on Day 1 (beginning of the assay) and at Day 6 (prior to $^3\text{HTdR}$ injection).

Proliferation assay: On Day 6 each mouse was intravenously injected via the tail vein with 250 μL of sterile PBS (phosphate buffered saline) containing approximately 20 μCi of $^3\text{HTdR}$ using a gauge 25G1" hypodermic needle with 1 mL sterile syringe. Once injected, the mice were left for 5 hours.

Five hours after intravenous injection, the mice were killed by CO_2 asphyxiation. The draining auricular lymph nodes were excised by making a small incision on the skin between the jaw and sternum, pulling the skin gently back towards the ears and exposing the lymph nodes. The nodes were then removed using forceps and the carcasses discarded. The nodes of mice from each test group was pooled and collected in separate Petri dishes containing a small amount (1-2 mL) of PBS to keep the nodes wet before processing.

A single cell suspension (SCS) of pooled lymph node cells (LNCs) were prepared and collected in disposable tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer using the plunger of a disposable syringe. The cell strainer was washed with PBS (up to 10 mL). Pooled LNCs were pelleted with a relative centrifugal force (RCF) of 190 x g (approximately) for 10 minutes at 4 °C. After centrifugation supernatants were discarded. Pellets were gently resuspended and 10 mL of PBS was added to the tubes. The washing step was repeated twice. This procedure was repeated for each group of pooled lymph nodes.

After the final washing step, the suspensions were centrifuged and the supernatants were removed leaving a small volume (<0.5 mL) of supernatant above each pellet. Each pellet was gently agitated before suspending the LNCs in 3 mL of 5 % TCA (trichloroacetic acid) for precipitation of macromolecules. After incubation with 5 % TCA at 2-8 °C overnight (approximately 18 hours) precipitate was recovered by centrifugation at 190 x g for 10 minutes at 4 °C, and supernatants were removed and pellets were resuspended in 1 mL of 5 % TCA solution and dispersed using an ultrasonic water bath. Each precipitate was transferred to a suitable sized scintillation vial with 10 mL of scintillation liquid and thoroughly mixed. The vials were loaded into a β -scintillation counter and $^3\text{HTdR}$ incorporation was measured for up to 10 minutes per sample. The β -counter expressed the $^3\text{HTdR}$ incorporation as the number of radioactive disintegrations per minute (DPM). Similarly, background radiation levels were also measured in two 1 mL aliquots of 5 % TCA.

Statistics / Data evaluation: In the absence of any positive results, the statistical analysis of the data was not performed.

DPM was measured for each pooled group of nodes. The measured DPM values were corrected with the background DPM value ("DPM"). The results were expressed as "DPN" (DPM divided by the number of lymph nodes) following the industry standard for data presentation.

A stimulation index of 3 or greater is the criteria for defining a positive result.

The test item is regarded as a sensitiser if both of the following criteria are fulfilled:

- That exposure to at least one concentration resulted in an incorporation of $^3\text{HTdR}$ at least 3-fold or greater than recorded in control mice, as indicated by the stimulation index.
- The data are compatible with a conventional dose response, although allowance must be made (especially at high topical concentrations) for either local toxicity or immunological suppression.

Results and discussion

Clinical observation: No mortality or signs of systemic toxicity were observed during the Main Study. No cutaneous reactions were observed at the site of the treatment in any treatment groups.

Body weight: No treatment related effects were observed on body weight.

Proliferation assay: Appearance of the lymph nodes was normal in the negative control group and in the test item treated groups. Larger than normal lymph nodes was observed in the positive control group.

No mortality, cutaneous reactions or signs of toxicity were observed in the positive control group. A significant lymphoproliferative response (stimulation index value of 12.2) was noted for α -Hexylcinnamaldehyde in this experiment. The results of the positive control group demonstrated the appropriate performance of the assay.

Conclusion by the Notifiers

In conclusion, under the conditions of the present assay, glyphosate technical tested in a suitable vehicle, was shown to have no skin sensitisation potential (non-sensitiser) in the Local Lymph Node Assay.

Comment by RMS:

The study is considered acceptable and no evidence for skin sensitisation potential of the test substance was observed.

B.6.3 Short-term toxicity (Annex IIA 5.3)

Introduction into this chapter by the RMS

Those short-term studies are reported in detail in this section that were not mentioned in the original DAR (1998, ASB2010-10302) because they either had not been submitted for previous EU evaluation or were conducted more recently. Using the GTF dossier as basis, study descriptions were amended where necessary and each of these “new” studies was commented by RMS. Redundant parts were deleted. The structure of the document was changed. Some summary tables have been included and, where available, studies with formulations are also mentioned now in separate sub-sections.

Most of the previously known studies from the 1998 DAR may be still used for risk assessment purposes. They were all subject to re-evaluation with regard to quality and reliability. For the study design and their results, however, we refer to the old DAR.

Overall evaluation is presented in Volume 1, including tables in which the (new and previously known) valid oral subchronic studies in rats and dogs are summarised.

B.6.3.1 Subacute studies (all routes)

B.6.3.1.1 Subacute oral studies in rats and dogs

No new sub-acute oral toxicity studies with glyphosate have been run since the previous evaluation was performed in the late 1990ies. The five studies mentioned in the old DAR were re-evaluated by the RMS. The studies by [REDACTED] (1989, TOX9552351) and by [REDACTED] (1991, TOX9551095, Z102035, Z102043) in rats were still considered acceptable although the latter one had the apparent deficiency that sexes were combined for reporting of hematological and clinical chemistry parameters and organ weights in the summary tables. However, raw data that may be easily found in the study report allowed separate assessment. Of a third study by [REDACTED] (1989, TOX9552352), at least “part B” in which a limit dose of 1000 mg glyphosate/kg bw was administered daily for two weeks to dogs was also considered acceptable whereas part A was difficult to interpret due to a very unusual design with increasing doses.

The pilot studies by [REDACTED] (1978, TOX9552348) in mice and of [REDACTED] (1989, TOX9552350) in rats are not acceptable due to serious reporting deficiencies.

The rather small number of valid subacute studies by the oral route is not considered a data gap because there is quite a lot of fully acceptable 90-day studies in all relevant species.

Studies with formulations

A study [REDACTED] (1982, TOX9552349) in dogs does also not completely comply to modern standards and may not be used for risk assessment of glyphosate but is of interest because higher toxicity of a formulation as compared to the active ingredient was elucidated. Strong gastrointestinal irritation became apparent when the isopropylamine (IPA) salt of glyphosate (MON 0139) was administered to Beagle dogs. Vomiting and diarrhea together with a reduction in body weight gain were observed at a dose level of 625 mg/kg bw and above. A dose of 312.5 mg/kg bw given for five days still caused a slight decrease in food consumption. Toxicity of isopropylamine alone was also tested in this experiment revealing strong irritation of gastric and esophageal mucosa at a much lower dose of 72 mg/kg bw upon

single and repeated administration suggesting that these effects were most probably not or only partly attributable to glyphosate itself.

B.6.3.1.2 Subacute inhalation studies (rat)

Only one study on subacute inhalative toxicity (14 days) in rodents (██████████ 1985, TOX9551952) is available and was reviewed in the 2001 EU evaluation. The previous review concluded no treatment-related effects were observed and the NOEL (more correct NOEC) was 3.8 mg/L. However, from a today's regulatory point of view, the study must be considered unacceptable due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material.

Studies with formulations

A four-week inhalation study with the formulation MON 2139 (██████████ 1983, TOX2002-694) suggesting some effects at rather low concentrations is reported and evaluated in the Appendix on formulations containing ██████████ as surfactants (see B.6.13).

B.6.3.1.3 Subacute percutaneous toxicity studies (rabbits and rats)

There was only one new study submitted for this evaluation, *i.e.*, a 21-day dermal study on rats by ██████████ (1996, ASB2012-11461) that is reported in the following in detail. A second study not mentioned in the previous DAR (██████████ 2012, ASB2012-11459) was originally allocated to this section by GTF but is now reported under B.6.12 since its focus was on dermal absorption (*in vitro*, rabbit skin) and not on toxicity.

For details regarding the studies that were reviewed during the 2001 EU evaluation, we refer to the original DAR (1998, ASB2010-10302). Re-evaluation by the RMS revealed that the study on rats by ██████████ (1993, TOX9552367) and those by ██████████ (1982, TOX9552366) and ██████████ (1994, TOX9650151) on rabbits may be still considered acceptable. In contrast, quality assessment of a further study in rabbits (██████████ 1985, TOX9551951) was downgraded to “not acceptable” (from “supplementary” in the old DAR) because of reporting deficiencies (batch and purity lacking). The absence of statistical analysis may be explained by the very low number of animals (3 per sex and dose) but this small group size is one more reason for doubts on the reliability of this study.

New subacute dermal toxicity study on rats (██████████ 1996)

Reference:	IIA, 5.3.7/04
Report:	██████████ 1996 Glyphosate Acid: 21 Day Dermal Toxicity Study In Rats ██ Data owner: Syngenta Report No.: ██████████/P/4985 Date: 1996-06-24 not published, ASB2012-11461
Guidelines:	OECD 410 (1981): OPPTS 870.3200 (1998): 87/302/EEC B.28 (1988)
Deviations:	None

GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1996-01-09 to 1996-06-24

Materials and methods

Test Material: Glyphosate acid
 Description: Technical, white solid
 Lot/Batch number: P24
 Purity: 95.6 % w/w a.i
 CAS#: Not reported
 Stability of test compound: Not reported
 Vehicle and/or positive control: Deionised water
 Test Animals:
 Species: Rat
 Strain: Alpk:APfSD
 Age/weight at dosing: 6 - 8.5 weeks / males 214-249 g, females 193-227 g
 Source: [REDACTED]
 Housing: Individually, in cages on multiple rat racks suitable for animals of this strain and weight range expected during the course of the study.
 Acclimatisation period: At least 5 days
 Diet: Diet (PCD) supplied by Special Diet Services Limited, Witham, Essex, UK *ad libitum*
 Water: Mains water *ad libitum*
 Environmental conditions: Temperature: 21 ± 2 °C
 Humidity: 40-70 %
 Air changes: At least 15 changes/hour
 Photoperiod: 12 hours light/12 hours dark

Study design and methods

In-life dates: Start: 10 January 1996 End: 1 February 1996

Animal assignment: The study was divided into ten (randomised blocks), each containing one cage per treatment group. The animals were randomly allocated to groups as shown below:

Table B.6.3-1: Study design ([REDACTED] 1996 ASB2012-11461)

Test group	Dose level of glyphosate acid (mg/kg/day)	# male	# female
Control	0	5	5
Low	250	5	5
Mid	500	5	5
High	1000	5	5

Preparation and treatment of animal skin:

Sixteen to twenty-four hours before application of the test substance, the hair was removed with a pair of veterinary clippers from an area, approximately 10 cm x 5 cm, on the dorso-lumbar region of each animal. The rats were dosed dermally and the amount applied was calculated for each animal according to its weight at the time of dosing. The paste covered by a gauze patch (approximately 7 cm x 7 cm x 4-ply) was applied to the shorn back of each animal and was kept in contact with the skin for approximately 6 hours using an occlusive dressing. The gauze patch was covered by a patch of plastic film (7 cm x 7 cm) and was held in position using adhesive bandage (25 cm x 7.5 cm). This was secured by two pieces of PVC tape (approximately 2.5 cm x 20 cm) wrapped around the animal. The control animals were treated in a similar manner except that deionised water only was used. The rats were dosed sequentially in group order at approximately the same time each day.

At the end of each 6-hour contact period, the dressings were carefully removed. The skin, at the site of application, was cleansed using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

A total of 15 six-hour applications was made during a period of 21 days. During this time there were three two-day periods when the animals were not dosed. Following each application there was an 18-hour 'rest' period during which the animals were fitted with plastic collars to prevent oral contamination.

Observations:

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. Detailed clinical observations were recorded daily and after decontamination. Cage-side observations were also made as soon as possible after dosing, and towards the end of the working day.

Body weight: The body weight of each rat was recorded daily, immediately prior to application of the test substance where applicable and prior to termination on day 22.

Food consumption and test substance intake: Food consumption was recorded continuously throughout the study for each rat and calculated as a weekly mean (g food/rat/day).

Haematology and clinical chemistry: Blood was collected at termination, by cardiac puncture and the following parameters were examined:

Haemoglobin	mean cell haemoglobin concentration
Haematocrit	platelet count
red blood cell count	total white cell count
mean cell volume	differential white cell count
mean cell haemoglobin	blood cell morphology
red cell distribution width	prothrombin time
activated partial thromboplastin time	

Clinical chemistry: Blood was collected at termination, by cardiac puncture and the following parameters were examined:

Urea	alkaline phosphatase activity
Creatinine	aspartate aminotransferase activity
Glucose	alanine aminotransferase activity
Albumin	gamma-glutamyl transferase activity
total protein	calcium
Cholesterol	phosphorus (as phosphate)
Triglycerides	sodium
total bilirubin	potassium
creatine kinase activity	chloride

Investigations *post mortem*:

Macroscopic examination: All animals were examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands	liver
Kidneys	testes

Paired organs were weighed together.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

gross lesions including masses	adrenal gland*
testis*	epididymis*
Kidney	treated skin
Liver	untreated skin

* Tissues marked were stored but not examined microscopically

Microscopic examination: All selected tissues (see above) processed from the control and the group receiving 1000 mg glyphosate acid/kg/day, together with macroscopic abnormalities from these groups, were examined by light microscopy.

Statistics:

Haematology, clinical chemistry, organ weights and weekly food consumption were analysed using Analysis of variance. Body weights, on initial (day 1) body weight, organ weights on final body weight were analysed using analysis of covariance. All data were analysed using SAS (1989).

Results and discussion

Mortality: There were no mortalities.

Clinical observations: There were no significant signs of toxicity at any dose level of glyphosate acid. Generally the clinical findings observed were consistent with those

commonly seen in dermal studies as a consequence of bandaging and were considered not to be related to treatment with glyphosate acid.

Bodyweight and weight gain: There were no effects due to treatment with glyphosate acid on bodyweight at any dose level.

Food consumption: There were no effects due to treatment with glyphosate acid on bodyweight at any dose level.

Haematology: A minimal statistically significant increase in haemoglobin levels was observed in females dosed at 1000 mg glyphosate acid/kg/day. A statistically significant decrease compared with control was seen in red cell distribution width in females dosed at 250 and 1000 mg glyphosate acid/kg/day. In the absence of any adverse effects on the red cell parameters, these minor changes are considered not to be of toxicological significance.

Table B.6.3-2: Intergroup comparison of selected haematology parameters (Pinto, 1996 ASB2012-11461)

Parameter	Dose level of glyphosate acid (mg/kg/day)							
	Males				Females			
	0	250	500	1000	0	250	500	1000
Haemoglobin	15.2	15.3	15.3	15.0	13.9	13.7	14.1	14.6*
Red cell distribution width	13.1	12.9	12.6	13.4	13.8	12.4**	13.0	12.6*

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

Blood clinical chemistry: Females dosed at 1000 mg glyphosate acid/kg/day showed a minimal but statistically significant increase in plasma urea levels, but there were no differences seen in the plasma creatinine levels. This minimal change in urea was considered not to be of toxicological significance. A minimal but statistically significant decrease in plasma triglycerides was observed in males dosed at 500 mg glyphosate acid/kg/day and as this did not form part of a dose response relationship was considered not to be treatment related.

Table B.6.3-3: Intergroup comparison of selected clinical chemistry parameters (Pinto, 1996 ASB2012-11461)

Parameter	Dose level of glyphosate acid (mg/kg/day)							
	Males				Females			
	0	250	500	1000	0	250	500	1000
plasma urea	8.4	8.2	8.5	8.1	7.6	7.7	6.9	8.6*
plasma triglycerides	1.27	1.01	0.87*	1.27	0.70	0.66	0.69	0.76

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

Sacrifice and pathology:

Organ weights: Testes weights were slightly but statistically significantly decreased at 500 mg glyphosate acid/kg/day, due to one animal having a very low weight recorded. There were no effects due to treatment with glyphosate acid in the other organs weighed.

Macroscopic findings: A small number of lesions were observed, none of which was related to treatment.

Microscopic findings: A small number of common spontaneous lesions were observed, none of which was related to treatment.

Conclusion by the Notifiers

There was no evidence of systemic toxicity or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg/day.

The no observed adverse effect level (NOAEL) for systemic toxicity and dermal irritation was considered to be 1000 mg glyphosate acid/kg/day in both sexes.

RMS comments:

The study is considered acceptable even though most dermal studies in rats include a treatment period of four weeks while rabbits are administered a test substance via the skin usually for 21 days. We agree with the conclusions including setting of the NOAEL for both systemic effects and dermal irritation at the limit dose of 1000 mg/kg bw.

Studies with formulations

Not available.

B.6.3.2 Subchronic toxicity (oral)

B.6.3.2.1 90-day studies in rats

A total of eight valid studies of this type were used for this evaluation of which 5 had been subject to the previous one (DAR, 1998, ASB2010-10302) yet. Re-evaluation of the studies by [REDACTED] (1987, TOX9552362), [REDACTED] (1989; TOX9552364) and Eadie (1989, TOX9551821) from the old DAR revealed that all these may be still considered fully acceptable even though in the latter one the actual mean dietary intake was not calculated.

In contrast, for the study by [REDACTED] (1993; TOX9650149), former assessment as “acceptable” was changed by the RMS into “supplementary” because the batch number but no purity of the test material was given. This same holds true for a study by [REDACTED] (1992, TOX9551096) in which reporting deficiencies were noted. Mean dietary intake was calculated in an amendment but not for the recovery group and no information was given which of the salivary gland had been examined histologically. Apparently, no additional control group was employed for the recovery part.

Two more supplementary studies mentioned in the original DAR were not included by the RMS into the re-evaluation. The study by [REDACTED] (1981, TOX9650152, in the former DAR erroneously cited as “anonymous”) may be considered as sufficiently replaced by a more recent one ([REDACTED] 1993, TOX9650149) that had been commissioned by the same company. The study by [REDACTED] (1990, TOX9500266) was run soon after the more comprehensive by [REDACTED] (1989, TOX9551821) in the same laboratory and the same rat strain. The same dose levels were used and no effects were noted up to the highest dose of 7500 ppm. Thus, this study would not contribute any new information to overall assessment.

One further study ([REDACTED] 1985, TOX9551822) was briefly described in the previous DAR and regarded therein as “supplementary” but is now considered not acceptable due to major reporting deficiencies. In addition, absence of statistical analysis was noted.

In addition, three new feeding studies in rats ([REDACTED] 1996, TOX2000-1990; [REDACTED] 1996, ASB2012-11451; [REDACTED] 1995, ASB2012-11452) were submitted that had not been

reviewed on EU level before. Subsequent to the summary table, they are reported in detail, with comments of the RMS on bottom of the respective study. For details regarding studies reviewed during the 2001 EU evaluation, we refer to the old monograph (DAR, 1998, ASB2010-10302).

1st new subchronic study in rats ([REDACTED] 1996)

Reference: IIA, 5.3.2/01

Report: [REDACTED] 1996 First Revision To Glyphosate Acid: 90 Day Feeding Study In Rats

[REDACTED]
Laboratory Report No.: CTL/P/1599

Data owner: Syngenta

Date: 1996-11-07, not published, TOX2000-1990

Guidelines: No guideline statement, but in accordance with OECD 408 (1998), OPPTS 870.3100 (1998), 2001/59/EC B.26 (2001)

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Materials and methods

Test material:

Identification: Glyphosate acid

Description: White solid

Lot/Batch #: P15

Purity: 97.4%

Stability of test compound: Not reported

Vehicle: Plain diet / none

Test animals:

Species: Rat

Strain: Alpk:APfSD

Source: [REDACTED]

Age: 36-38 days

Sex: Male and female

Weight at dosing: ♂ 98-170 g; ♀ 96-140 g

Acclimation period: Approximately 1 week

Diet/Food: CTI diet, *ad libitum*, (except during collection of urine samples)

Water: Mains water, *ad libitum*, (except during collection of urine samples)

Housing: 4/cage, sexes separately in stainless steel cages 34.0 x 37.5 x 20.3 cm giving a floor area of 1275cm²

Environmental conditions: Temperature: 21 ± 2 °C
Humidity: 36 - 60 %
Air changes: ≥ 15 /hour
12 hours light/dark cycle

Study design and methods:
In life dates: 1996-02-25 to 1996-05-25

Animal assignment and treatment:
The study consisted of one control and three treatment groups each containing twelve male and twelve female rats.

Table B.6.3-4: Study design

Test group	Dietary concentration (ppm)	Males	Females
Control	0	12	12
Low	1000	12	12
Mid	5000	12	12
High	20000	12	12

The study was divided into six single-sex replicates (randomised blocks). Each replicate consisted of four cages, one per treatment group. The animals were randomly allocated to cages.

The experimental diets were made in 60 kg batches by adding the appropriate amount of glyphosate acid to the diet using dry premixes. Samples from all dietary levels (including controls) were taken from both batches prepared for the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was determined by analysing samples from the low and high dose levels from the first batch of diet. The chemical stability of glyphosate acid in diet was determined at the highest and lowest dose levels at 1, 4, 6 and 10 weeks after preparation. Analysis was by high performance liquid chromatography (HPLC).

Mortality: Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations: A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before of the beginning of the treatment period and then once a week until the end of the study.

Body weight: The bodyweight of each animal was recorded immediately before feeding of the experimental diets commenced and then on the same day, where practicable, of each subsequent week until termination. The body weight determination was done on the same day on which the detailed clinical examination was performed.

Food consumption and utilisation: Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage. The food utilisation value per cage was calculated as the bodyweight gained by the rats in the cage per 100 g of food eaten.

Ophthalmoscopic examination: The eyes of all animals from the control group and the 20000 ppm glyphosate acid dose level group were examined in the week prior to termination, using an indirect ophthalmoscope and a mydriate to dilate the pupil.

Haematology and clinical chemistry: At termination, all surviving rats were bled by cardiac puncture and the blood samples were collected both in tubes containing EDTA as anticoagulant and also in tubes containing 0.11M trisodium citrate. These samples were submitted for haematological examination and the following parameters measured: haemoglobin, haematocrit, red blood cell count, MVC, MCHC, MCH, kaolin-cephalin times, thrombocytes, leucocytes, differential white cell count, red blood cell morphology, prothrombin time.

For clinical chemistry analysis blood samples were collected by tail vein bleeding at week 4 of the study and by cardiac puncture at termination (week 13). The blood was collected in lithium heparinised tubes and the following parameters measured: glucose, urea, total protein, albumin, total cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT).

Urinalysis: Urine samples were collected over an 18 hour (approximately) period from all rats during week 13 (the week prior to termination). During urine collection, the rats were individually housed in metabolism cages and denied access to food and water. The following parameters were measured: volume, pH, specific gravity, proteins, glucose, ketones, and urobilinogen.

Sacrifice and pathology: On completion of the treatment period, all surviving animals were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: adrenals, brain, epididymides, heart, kidneys, liver, and testes. Paired organs were weight together.

Tissue samples were taken from the following organs and preserved in buffered formalin: all gross lesions, adrenals, aorta, bone marrow (femur), brain, caecum, colon, duodenum, epididymides, eyes (stored), Harderian gland (stored), heart, ileum, jejunum, kidneys, larynx (stored), liver, lungs, lymph nodes (cervical and mesenteric), mammary gland, ovaries, oviducts, pancreas, pituitary gland, prostate, rectum, salivary glands, seminal vesicles, spinal cord, sciatic nerve, skin, spleen, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus (with cervix), voluntary muscle and nasal cavity.

Following fixation, all tissues from the control and 20000 ppm glyphosate acid groups (except those stored) were processed by standard methods, embedded in paraffin wax, sectioned at 5µm, stained with haematoxylin and eosin and examined by light microscopy. Liver, kidney, adrenals, lungs and abnormal tissues from animals fed 1000 ppm or 5000 ppm glyphosate acid were also processed to blocks and were examined histologically.

Statistics: All data were evaluated using analysis of variance (bodyweight gain from start of study, final bodyweight, haematology, clinical chemistry – blood and urine, total food consumption and utilisation, organ weights) and covariance (organ weights on terminal bodyweights) for each specified parameter using the GLM procedure in SAS (1982).

Results and discussion

Mortality: There were no mortalities.

Clinical observations: The incidence of clinical findings was low and none was unequivocally related to treatment. There was a low incidence of diarrhoea (during the second week of the study) in the group receiving 20000 ppm glyphosate acid. The faeces of both sexes at this dose level were observed to be paler than those of control or other test groups.

Body weight: No relevant differences in the mean body weight gain were noted between controls and animals given 1000 or 5000 ppm.

Body weight gain was reduced in male rats fed 20000 ppm glyphosate acid from the first week of the study. The body weights continued to diverge from control values as the study

progressed, and final body weights were approximately 8 % lower than those of controls (see Table B.6.3-5).

Table B.6.3-5: Intergroup comparison of bodyweight gain – selected timepoints from start of study

	Mean cumulative bodyweight gain (g)							
Timepoint	Initial	Week 1	Week 2	Week 4	Week 7	Week 10	Week 13	Final weight
Dose (ppm)	Males							
0	135	51.8	104.0	185.5	254.6	305.1	333.3	468.3
1000	140.3	54.3	106.3	187.5	253.4	304.1	327.0	467.3
5000	136.3	51.8	103.4	186.1	255.1	306.3	331.9	468.3
20000	134.5	45.1**	94.0*	166.9**	226.0**	272.0**	295.8**	430.3**
	Females							
0	121.3	26.6	47.3	81.6	112.8	130.8	143.3	264.6
1000	122.2	27.7	51.4	82.7	113.1	132.1	146.0	268.2
5000	121.3	25.9	50.2	82.9	110.0	129.5	138.4	259.8
20000	118.6	24.3	53.5*	83.3	115.1	132.7	142.5	261.1

* Statistically significant from controls, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant from controls, $p < 0.01$ (Student's t-test, 2-sided)

Food consumption and test substance intake: The food consumption of males fed 20000 ppm glyphosate acid was reduced from the fifth week of the study compared to control values but the reduction was small and did not attain statistical significance in any week. The food utilisation efficiency of males at this dose level was reduced throughout the study. The food consumption and food utilisation efficiency of males fed 1000 or 5000 ppm glyphosate acid and of females at all dose levels were similar to those of controls.

Table B.6.3-6: Intergroup comparison of food utilisation (g growth/100 g food)–selected timepoints from start of study

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Weeks	0	1000	5000	20000	0	1000	5000	20000
1-4	25.15	24.85	24.99	22.89	15.73	15.42	15.78	15.91
5-8	11.25	11.09	11.36	10.31	7.49	6.74	6.71	7.29
9-13	6.45	5.76	6.05	5.88	3.08	3.95	3.10	2.96
Overall (1-13)	13.59	13.30	13.44	12.54	8.28	8.34	8.14	8.25

* Statistically significant from controls, $p < 0.05$ (Student's t-test, 2-sided)

Calculated mean test compound intakes are presented in the following table.

Table B.6.3-7: Overall mean test compound intake

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Weeks	0	1000	5000	20000	0	1000	5000	20000
1-13	0	81.33	413.5	1612	0	90.42	446.9	1821

* Statistically significant from controls, $p < 0.05$ (Student's t-test, 2-sided)

Ophthalmoscopic examination: There were no test substance-related ophthalmological findings at the end of the treatment period. The small incidence of findings recorded was within the normal background incidence for rats of this age and strain.

Haematology and clinical chemistry

Haematology: There were no treatment-related effects noted in any dose group.

Blood clinical chemistry: The plasma activities of alanine transaminase (ALT) and alkaline phosphatase (ALP) were increased in both sexes fed 20000 ppm glyphosate acid throughout the study. Plasma aspartate transaminase activity was increased in females fed 20000 ppm glyphosate acid at week 4 only.

Plasma ALT activity was also increased in males receiving 5000 ppm glyphosate acid at weeks 4 and 13 and in females at week 4 only.

The plasma ALP activities of males receiving 5000 or 1000 ppm glyphosate acid were marginally increased. These increases were not dose-related and for the 1000 ppm glyphosate acid group were attributed to the high values in 3 out of 12 males. These marginal differences from the control group are considered to be of doubtful significance and not to be treatment-related.

Plasma urea levels were marginally decreased in both sexes at week 13 and in males at week 4 in the 20000 ppm glyphosate acid group.

Males receiving glyphosate acid showed marginal reductions in plasma glucose levels at week 4 but not at week 13. Females at 20000 ppm glyphosate acid showed a slight increase in this parameter at week 13 only.

Plasma cholesterol levels were unaffected by treatment with glyphosate acid. Plasma triglyceride levels were slightly reduced in males receiving 20000 ppm glyphosate acid at both weeks 4 and 13, the effect being greater at week 13.

Both males and females receiving glyphosate acid showed marginal reductions in plasma albumin and total protein. The changes were not consistent, showed no dose-response relationship and are therefore considered to be of dubious significance.

Table B.6.3-8: Intergroup comparison of selected clinical chemistry parameters

		Dietary concentration of glyphosate acid (ppm)							
		Males				Females			
Parameter	Week	0	1000	5000	20000	0	1000	5000	20000
ALAT	4	61.0	66.8	76.0**	83.8**	47.3	50.8	57.7*	73.8**
	13	51.9	52.3	62.3*	65.2**	45.0	45.2	46.2	55.0**
ALP	4	273	326**	320*	411**	188	199	212	309**
	13	148	159	176*	215**	91	94	99	140**
ASAT	4	62.8	67.0	69.1	68.5	56.0	57.0	57.5	64.8**
Urea	4	47.0	45.8	44.6	43.6*	45.8	45.3	46.8	44.2
	13	41.9	39.9	40.0	37.7*	40.6	40.1	42.1	35.9*
Glucose	4	143	133*	132*	128**	141	146	142	136
	13	191	186	208	197	182	183	183	208**
Triglycerides	4	151	145	147	136	80	75	88	84
	13	153	157	144	120**	72	74	77	77
* Statistically significant from controls, p<0.05 (Student's t-test, 2-sided)									
** Statistically significant from controls, p<0.01 (Student's t-test, 2-sided)									

Urine analysis: There were no treatment-related findings.

Necropsy

Organ weights: Absolute heart weight of top dose males was reduced compared to controls but the reduction reflected the reduced bodyweight. There were no other differences in organ weights which were considered to be related to treatment

Gross pathology: A small number of lesions were observed, none of which was related to treatment.

Histopathology: There were no histopathological findings related to treatment. The incidence of findings was low and, with one exception, of a type commonly found in rats of this strain and age. An uterine leiomyosarcoma was seen in a female fed 5000 ppm glyphosate acid. Whilst the occurrence of a malignant tumour of smooth muscle in the uterus of a young rat is unusual, this isolated finding in an intermediate dose group is considered not to be related to treatment.

Conclusion by the Notifiers

Under the experimental conditions of the study and taking into account the reduced growth (males only) and biologically insignificant changes in clinical chemistry parameters at the high dose-level, the No Observed Adverse Effect Level (NOAEL) is considered to be 5000 ppm (equivalent to 414 and 447 mg/kg bw/day for males and females, respectively).

RMS comments

This study is considered acceptable and the conclusions, including setting of the NOAEL, are agreed with. The significant changes in clinical chemistry parameters at the mid dose level in males might be indicative of a weak effect on the liver, however, since they were not accompanied by histological lesions and/or liver weight increase, are not regarded as adverse. In addition to the study description above, it should be noted that, as in previous studies with glyphosate, urine pH was significantly decreased in top dose males and in mid and high dose females. From the study report it seems that salivary glands were taken but neither weighed nor examined microscopically although they were a target organ in other studies.

2nd new subchronic study in rats (██████████ 1996)

Reference: IIA, 53.2/03

Report: ██████████ 1996,
Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary)
Toxicity Study In The Rat

██████████ Project No.: 434/016

Date: 1996-07-16, unpublished, ASB2012-11451

Guidelines: JMMAF 59 NohSan No. 4200

(Data from the study report is equivalent to OECD 408.)

Deviations: FOB was not conducted. Some mandatory organs were not weighed/examined.

GLP: yes

Acceptability: See RMS comment

Materials and methods

Test material:

Identification: Technical Glyphosate

Description: White powder

Lot/Batch #: H95D 161 A

Purity: 95.3 %

Stability of test compound: No data given in the report.

Vehicle: Plain diet

Test animals:

Species: Rats

Strain: Sprague-Dawley (CD)

Source: [REDACTED]

Age: 6-7 weeks

Sex: male and female

Weight at dosing: ♂ 175 - 218 g; ♀ 145 - 195 g

Acclimation period: 7 days

Diet/Food: Rat and Mouse SQC Ground Diet No.1 (Special Diets Services Limited, Witham, Essex, UK), *ad libitum*

Water: tap water, *ad libitum*

Housing: In groups of up to four by sex in polypropylene grid-floor cages.

Environmental conditions: Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: 15/hour
12 hours light/dark cycle

Study design and methods:

In life dates: 1995-08-11 to 1996-01-30

Animal assignment and treatment:

In a 90 day feeding study, groups of 10 Sprague Dawley rats per sex received daily dietary doses of 0, 1000, 10000 and 50000 ppm (equivalent to mean achieved dose levels of male; 0, 79, 730 and 3706 mg/kg bw/day, female; 90, 844, 4188 mg/kg bw/day respectively) technical glyphosate in the diet.

Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.

The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for homogeneity and achieved concentration.

Clinical observations: A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded.

Body weight: Individual body weights were recorded on Day 0 (prior to treatment) and at weekly intervals thereafter. Body weights were also determined at necropsy.

Food consumption and compound intake: Food consumption was recorded once weekly for each cage group throughout the study.

Water consumption: Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Ophthalmoscopic examination: The eyes of all control and high dose animals were examined before administration of the test and control diets and before termination of treatment (during Week 12). Examinations included observation of the anterior structures of the eye, pupillary and corneal blink reflex and, following pupil dilation with 0.5 % Tropicamide solution ("Mydriacyl" - Alcon Laboratories Ltd., Watford, Hertfordshire, UK), detailed examination of the internal structure of each eye using a direct ophthalmoscope.

Haematology and clinical chemistry: Haematological and blood chemical investigation were performed on all animals from each test and control group at the end of the study (Day 90).

Urinalysis: Urinalytical investigations were performed on all animals during Week 12. Urine samples were collected overnight by housing the rats in metabolism cages. Animals were maintained under conditions of normal hydration during collection but without access to food.

Sacrifice and pathology: All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: adrenals, brain, gonads, heart, kidneys, liver, pituitary and spleen.

Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (at three levels), caecum, colon, duodenum, eyes, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

Statistics: Absolute and relative organ weights, haematological and blood chemical data were analysed by one way analysis of variance incorporating 'F-max' test for homogeneity of variance. Data showing heterogeneous variances were analysed using Kruskal-Wallis non-parametric analysis of variance and Mann Whitney U-Test.

The levels of probability chosen as significant were $p < 0.001^{***}$, $p < 0.01^{**}$ and $p < 0.05^{*}$.

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:

1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of 1 or greater.
2. Kruskal-Wallis one way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

The levels of probability chosen as significant were $p < 0.001^{***}$, $p < 0.01^{**}$, $p < 0.05^{*}$ and $p < 0.1^{(*)}$.

Results and discussion

Mortality: No deaths occurred during the study.

Clinical observations: Animals of both sexes treated with 50,000 ppm showed soft faeces and diarrhoea from Day 4 which continued throughout the study period.

The remaining observable sign of generalised fur loss was noted in one male and two females treated with 10,000 and 1,000 ppm respectively. This is a commonly reported incidental finding in laboratory maintained rats that, in the absence of any dose-related response, is of no toxicological significance and unrelated to treatment with the test material.

Body weight: Animals of both sexes treated with 50,000 ppm showed a reduction in bodyweight gain over the first four weeks of treatment when compared with controls (see Table B.6.3-9). Female bodyweight development recovered as the study progressed and was comparable with the control group by the end of the treatment period. Male individuals showed only a partial recovery with body weight gain remaining slightly lower than the control group values during the subsequent weeks of treatment.

Body weight development was unaffected by treatment with the test material at the remaining dose levels.

Table B.6.3-9: Group mean weekly bodyweights and standard deviations (sd)

Dietary concentration		Bodyweight (g) at Day													
(ppm)		0	7	14	21	28	35	42	49	56	63	70	77	84	90
		Males													
0	mean	206	269	315	354	382	411	444	457	488	508	523	537	536	551
	sd	8	12	17	24	33	38	45	44	49	52	55	58	56	58
1000	mean	199	260	309	350	377	400	427	446	470	485	497	513	516	528
	sd	11	14	19	21	24	26	30	31	32	32	35	37	36	37
10000	mean	200	257	303	338	364	393	414	429	454	470	483	494	495	506
	sd	12	12	15	21	25	30	35	35	38	38	38	40	39	43
50000	mean	198	215	247	268	283	306	329	335	356	369	382	394	395	408
	sd	8	8	15	21	26	31	33	38	41	43	43	44	42	44
		Females													
0	mean	173	197	214	232	243	256	269	276	284	291	295	306	304	307
	sd	9	11	12	15	16	18	20	19	20	21	24	25	25	27
1000	mean	173	199	218	238	249	261	272	280	286	292	300	308	304	313
	sd	10	13	14	16	16	17	18	19	18	18	19	21	20	20
10000	mean	166	184	201	217	226	237	246	256	262	267	272	277	276	282
	sd	14	18	21	25	24	26	27	27	27	27	27	29	28	29
50000	mean	173	183	197	214	219	231	240	246	251	260	265	271	267	273
	Sd	11	12	14	15	14	18	21	21	20	23	23	26	22	25

Food consumption: Animals of both sexes treated with 50,000 ppm showed a reduction in both dietary intake and food efficiency over the first four weeks of treatment when compared with controls (see Table B.6.3-10). Female food consumption and efficiency recovered as the study progressed and was comparable with control values by the end of the treatment period. Male food consumption however, remained adversely affected during the subsequent weeks of treatment. A similar prolonged effect on food efficiency was not evident during the same period as male body weight gain demonstrated a partial recovery over the corresponding weeks.

Dietary intake and food efficiency were unaffected by treatment with the test material at the remaining dose levels and were comparable with controls.

Table B.6.3-10: Group mean weekly food consumption

Dietary concentration (ppm)	Mean food consumption (g/rat/week)												
	1	2	3	4	5	6	7	8	9	10	11	12	13*
	Males												
0	201	199	204	212	208	218	208	222	224	223	24	192	185
1000	200	205	213	211	205	210	211	217	204	219	214	191	180
	(0)	(3)	(4)	(0)	(-1)	(-4)	(1)	(-2)	(-9)	(-2)	(0)	(-1)	(-3)
10000	187	193	199	204	202	198	201	205	211	211	201	185	179
	(-7)	(-3)	(-2)	(-4)	(-3)	(-9)	(-3)	(-8)	(-6)	(-5)	(-6)	(-4)	(-3)
50000	122	183	178	177	183	182	168	187	189	193	188	174	171
	(-39)	(-8)	(-13)	(-17)	(-12)	(-17)	(-19)	(-16)	(-16)	(-13)	(-12)	(-9)	(-8)
	Females												
0	140	131	171	153	149	149	152	152	151	147	155	139	128
1000	143	146	152	156	158	163	157	159	152	154	161	141	137
	(2)	(+11)	(-11)	(2)	(6)	(9)	(3)	(5)	(1)	(5)	(4)	(1)	(7)
10000	123	135	142	144	143	140	143	146	143	143	142	133	131
	(-12)	(3)	(-17)	(-6)	(-4)	(-6)	(-6)	(-4)	(-5)	(-3)	(-8)	(-4)	(2)
50000	128	143	131	148	167	157	148	151	151	151	161	139	139
	(-9)	(9)	(-23)	(-3)	(23)	(5)	(-3)	(-4)	(0)	(3)	(4)	(0)	(9)

() - % change compared to control group

* - Week 13 comprises six days only

Water consumption: There were no treatment-related effects on water consumption for either sex noted during the study.

Ophthalmoscopic examination: No treatment-related ocular effects for either sex noted were detected during the study.

Haematology: No treatment-related effects were detected in the haematological parameters measured.

Blood chemistry: Animals of both sexes treated with 50,000 or 10,000 ppm showed a statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase (AP) when compared with controls (see Table B.6.3-11). A statistically significant increase in inorganic phosphorus and reduction in plasma creatinine were also evident amongst animals of both sexes treated with 50,000 ppm whilst females at this dose level showed statistically significant reductions in total plasma protein and albumin in comparison with controls.

There were no further treatment-related effects.

Table B.6.3-11: Group mean blood chemical values and standard deviations (sd)

Dietary concentration (ppm)		Ca ²⁺ (mmol/L)	AP (IU/L)	P (mmol/L)	Creatinine (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
		Males					
0	mean	2.74	373	2.23	0.61	—	—
	sd	0.06	101	0.22	0.03	—	—
1000	mean	2.77	404	2.22	0.62	—	—
	sd	0.07	115	0.16	0.05	—	—
10000	mean	2.66*	514*	2.32	0.59	—	—
	sd	0.09	106	0.28	0.04	—	—
50000	mean	2.64*	597***	2.46*	0.57*	—	—
	sd	0.10	150	0.22	0.04	—	—

Dietary concentration (ppm)		Ca ²⁺ (mmol/L)	AP (IU/L)	P (mmol/L)	Creatinine (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Females							
0	mean	2.78	230	1.70	0.69	7.63	3.90
	sd	0.11	38	0.33	0.07	0.45	0.23
1000	mean	2.76	261	1.65	0.69	7.64	3.87
	sd	0.05	71	0.21	0.04	0.29	0.13
10000	mean	2.70*	408***	1.76	0.65	7.41	3.82
	sd	0.07	123	0.23	0.04	0.45	0.20
50000	mean	2.56***	358**	2.12***	0.61**	6.86**	3.47***
	sd	0.10	90	0.15	0.05	0.82	0.39

— no significant changes

* significantly different from control group (p < 0.05)

** significantly different from control group (p < 0.01)

*** - significantly different from control group (p < 0.001)

Urinalysis

Animals of both sexes treated with 50,000 ppm showed an increased level of haemoglobin in the urine when compared with controls (see Table B.6.3-12). Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated at 50,000 ppm. This probably represents external contamination, possibly of faecal origin. There were no treatment-related changes detected at the remaining dose levels.

Table B.6.3-12: Urine findings

Dietary concentration (ppm)	Blood (haemoglobin)							
	Males				Females			
	-	+	++	+++	-	+	++	+++
0	8	0	1	1	10	0	0	—
1000	10	0	0	0	10	0	0	—
10000	7	2	1	0	10	0	0	—
50000	1	5	2	2	4	3	3	—

— - negative

+ - ca. $5-10 \times 10^6$ ery/L; ++ - ca. 50×10^6 ery/L; +++ - ca. 250×10^6 ery/L

Organ weights

Animals of both sexes treated with 50,000 ppm showed statistically significant increases in both relative liver and kidney weight when compared with controls (see Table B.6.3-13). There were no further direct effects of treatment.

Table B.6.3-13: Group mean organ weights and standard variations (sd)

Dietary concentration (ppm)		Relative organ weight (%)			
		Liver		Kidney	
		♂	♀	♂	♀
0	mean	2.9749	2.9734	0.5861	0.6516
	sd	0.2629	0.1558	0.0575	0.0523
1000	mean	2.8868	2.9093	0.5901	0.6257
	sd	0.2552	0.2146	0.0804	0.0375
10000	mean	2.8853	2.9801	0.6070	0.6454
	sd	0.3758	0.1556	0.0552	0.0532
50000	mean	3.2433*	3.1989*	0.6963***	0.7180*
	sd	0.2452	0.2098	0.0436	0.0707

* - significantly different from control group (p < 0.05)

*** - significantly different from control group ($p < 0.001$)

Necropsy

Macroscopic abnormalities were detected in the 50,000 ppm dose group with all animals showing enlarged and fluid-filled caecums whilst one female treated with 50000 ppm showed gaseous distension of the stomach at terminal kill.

There were no treatment-related macroscopic abnormalities detected at 10,000 or 1,000 ppm.

Histopathology

Treatment-related changes were observed in the caecum. Atrophy, characterised by flattening of the intestinal mucosa, was observed for five rats of both sexes dosed at 50,000 ppm ($p < 0.05$ for male rats) and for one male and two female rats receiving 10,000 ppm of the test material. The aetiology of this change is uncertain and may represent no more than a stretch atrophy of the mucosa resulting from caecal distension.

There were no further treatment-related changes.

Conclusion by the Notifiers

Dietary administration of the test material, technical glyphosate, to rats for a period of ninety consecutive days at dietary concentrations of up to 50,000 ppm resulted in treatment-related changes at 50,000 and 10,000 ppm. No such effects were demonstrated in the 1,000 ppm treatment group and the “No Observed Effect Level” was, therefore, considered to be 1000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females).

RMS comments

The study is considered acceptable and the conclusions drawn are supported. The data submitter/owner was not mentioned in the study description but is presumed to be Nufarm. According to the study report itself, the sponsor was Mastra Industries (Malaysia) that gave Nufarm access to this study.

The NOAEL of 79 mg/kg bw/day is agreed with. It must be kept in mind that, due to dose spacing, the margin between the low and mid dose level was very large. The LOAEL of 730 mg/kg bw/day was well above the NOAELs in other 90-day studies. Thus, despite the (relatively) low NOAEL, this study is not suitable to prove that toxicity of glyphosate was in fact higher than previously assumed. It must be also emphasised that the top dose level of 3700 mg/kg bw/day was indeed extremely high, even for this rather non-toxic compound.

Unfortunately, it is not clear from the report whether or not salivary glands (and if, which of them) were subject to histopathology.

Organ weight changes (including higher relative testis weight in addition to liver and kidney) at the top dose level are considered secondary to reduced body weight.

3d new subchronic study in rats [REDACTED] 1995)

Reference:

IIA, 5.3.2/04

Report:

[REDACTED] (1995) HR-001 : 13-week Subchronic Oral Toxicity Study in Rats.

Laboratory Report No.: [REDACTED] 94-0138

Data owner: Arysta LifeScience

Date: 1995-07-20

not published, ASB2012-11452

Guidelines:

Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA

FIFRA Guidelines Subdivision F, 1984; OECD 408 (1981)

Deviations: None
GLP: Yes
Acceptability: See RMS comment

Materials and methods

Test material: Glyphosate technical
 Identification: HR-001
 Description: White crystal
 Lot/Batch #: 940908-1 941209 T-941209
 Purity: 95.68 % 95.0 % 97.56 %
 Stability of test compound: 12/12/1994 19/12/1994 26/12/1994
 Vehicle: Plain diet / none
 Test animals:
 Species: Rat
 Strain: Sprague-Dawley Crj:CD
 Source: XXXXXXXXXX
 Age: 5 weeks
 Sex: Male and female
 Weight at dosing: ♂ 136-150 g, ♀ 109-121 g
 Acclimation period: 1 week
 Diet/Food: MF Mash (Oriental Yeast Co., Ltd.
 Water: Filtered and sterilized tap water, *ad libitum*
 Housing: 3/cage, sexes separately in stainless steel cages 31.0 x 44.0 x 20.3 cm
 Environmental conditions: Temperature: 24 ± 2 °C
 Humidity: 55 ± 15 %
 Air changes: 15/hour
 12 hours light/dark cycle

Study design and methods:

In life dates: 1994-12-06 to 1995-03-22

Animal assignment and treatment:

The test substance was incorporated into the basal effect diet and administered on a continuous basis in the basal diet to groups of 24 Sprague-Dawley rats (12 males + 12 females) for a period of 13 weeks. Dietary concentrations were 0, 3000, 10000 and 30000 ppm.

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level taken from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance.

Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality

Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations

Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight

Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weight were recorded for all animals before necropsy.

Food consumption and utilisation

Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumption by the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.

Group mean chemical intake (mg/kg/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.

Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Ophthalmoscopic examination

Ophthalmological examinations including observation with a haloen ophtalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13.

The following parts of the eye were inspected: eyeball, cornea, anterior chamber, pupil, iris, lens/vitreous body, fundus.

Haematology and clinical chemistry

After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomized under anesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.

The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC) and differential leukocyte count.

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.

The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ -glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), potassium (K) and chloride (Cl).

Urinalysis

At 13 week of treatment, all surviving animals were subjected to urinalysis. Fresh urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was determined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semiquantitatively analyzed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology

Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.

The following organs/tissues were taken: brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, pharynx, buccal mucosa of oral cavity, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

Statistics

All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologic parameters, blood chemical parameters, and organ weights).

Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

Results and discussion

Mortality:

No deaths were noted in the control and treated groups of either sex.

Clinical observations:

There were no abnormalities related to the treatment in the treated groups of either sex. In the 30 000 ppm group, one female showed a poor general condition including emaciation and decreased spontaneous motor activity. The poor general condition seemed to be caused by elongated incisor, malocclusion, or hepatorenal genetic lesions revealed by histopathology. Thus it was not considered to be treatment related.

Body weight:

In the 30 000 ppm group, body weights of males and females were slightly lower (about 5-10 % decrease in males and 5 % in females) than those in the control throughout the treatment period. Statistically significant decreases in their body weights were sporadically observed during the treatment period (weeks 3, 4 and 11 in males and weeks 10 and 11 in females) when compared to the control.

In the 10 000 and 3 000 ppm groups, body weight changes in males and females were comparable to the control throughout the treatment period.

Food consumption and test substance intake:

In the 30 000 ppm group, males and females showed significant decreases in food consumption at week 1 which were 9 and 14 % lower than that of the control, respectively. However, their food consumption were comparable to the control at week 2 and thereafter.

In the 10 000 no significant change was observed while in the 3000 ppm group, significant changes were sporadically observed during the treatment period in females during the weeks 6 and 7. The food consumption recovered from the week 8 up to the end of the study.

The overall food consumption by males and females was comparable to the control and there were no abnormalities considered treatment related.

The overall group mean chemical intakes averages, calculated from food consumption and nominal concentrations of the test substance, through the treatment period, were:

Table B.6.3-14: Mean daily intake of glyphosate in the study by Kinoshita (1995, ASB2012-11452)

Dose (ppm)	Chemical Intake (mg/kg bw/day)	
	Male	Female
3 000	168.4	195.2
10 000	569	637
30 000	1735	1892

Ophthalmoscopic examination

In the ophtalmological examination performed on all animals before the start of the treatment and on the animals of the control and 30 000 ppm groups at 13 weeks of treatment, no abnormalities were observed in either sex.

Haematology: Tere were no abnormalities in any group of either sex.

Blood clinical chemistry: In the 30 000 ppm group, females showed a significant increase in alkaline phosphatase (ALP) activity and a significant decrease in albumin (Alb). There were no abnormalities in males.

In the 10 000 and 3 000 ppm groups, there were no abnormalities in either sex.

Urine analysis: In the 30 000 ppm group, urine pH in males and females was significantly lower than that in the control. Urine protein showed a significant decrease in males and a decreasing trend in females. In addition, females showed a significantly higher urine volume than that of the control, but males showed a decreasing trend in urine volume as compared with the control.

In the 10 000 ppm group, urine, pH and protein in males were lower than those in the control. In females, no statistically significant change was observed in any parameter.

In the 3 000 ppm group, no statistically significant changes was observed in either sex.

Organ weights: In the 30 000 ppm group, both sexes showed significant increases in absolute and relative weights of the cecum (containing contents). In addition, females in this group also showed significant increases in relative weights of the brain and liver.

In the 10 000 ppm group, the absolute and relative weight of the cecum showed a statistically significant increase in males and increasing trend in females.

In the 3 000 ppm group, there were no abnormalities attributable to the treatment in either sex.

Gross pathology: In the 30 000 ppm group, distention of the cecum was observed in 9/12 males and 7/12 females with statistical significance. There were no other macroscopic abnormalities attributable to the treatment.

In the 10 000 ppm group, 3/12 males showed distention of the cecum, but there were no macroscopic abnormalities in females.

In the 3 000 ppm group, there were no macroscopic abnormalities attributable to the treatment in either sex.

Histopathology: Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment-related changes were not observed. One male in the 10 000 ppm group and one female in the 30 000 ppm group showed renal lesion (polycystic kidney) and hepatic lesions (bile ductal proliferation and cholangiectasis). It is generally regarded that these lesions were caused by genetic disorder and were not considered to be treatment-related.

Conclusion by the Notifiers

Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) is considered to be 3000 ppm (equivalent to 168.4 and 195.2 mg/kg bw/day for males and females, respectively).

RMS comments:

The study is considered acceptable and the proposed NOAEL is agreed with. Toxicity became apparent by the caecum findings and alterations in few clinical chemistry and urine parameters. The LOAEL of 569 mg/kg bw/day (10000 ppm) is higher than the NOAEL as established in other studies and, thus, does not contradict the previous assessment. From the study report, it became clear that submaxillary and sublingual salivary glands were histologically examined, without evidence of pathological changes. These glands were not weighed and parotid gland was not taken.

Published information

Glyphosate was tested in the 1980ies in U.S. National Toxicology Program (NTP) for oral subchronic toxicity (Chan and Mahler, 1992, TOX9551954). The following paragraph was partly copied from the previous DAR (1998, ASB2010-10302):

20 F344/N rats per sex and dose were fed glyphosate (supplied by Monsanto, approximately 99% pure) for 13 weeks at dietary levels of 0, 3125, 6250, 12500, 25000 or 50000 ppm. Ten rats/sex and group were used for evaluation of haematological and clinical chemistry parameters. All rats survived until the end of the study and there were no clinical signs of toxicity apart from diarrhea at the top dose level in both sexes. Body weight gain was markedly reduced in high dose males and slightly decreased in high dose females. There were some minor alterations in haematological and clinical chemistry parameters at least at the upper dose levels. Morphologic changes at necropsy were confined to parotid and

submandibular (submaxillary) salivary glands in both sexes. This “cytoplasmatic alteration” consisted of basophilic change and hypertrophy of acinar cells. The parotid gland was more affected. Here, the normal granular, eosinophilic staining cytoplasm of the acinar epithelial cells was replaced by basophilic and finely vacuolated cytoplasm. A NOEL could not be established since these lesions were observed already at the lowest dose level but not in the control groups. The degree of change showed a clear dose response. The outcome of this study shows that glyphosate is of low toxicity when administered orally over a period of 3 months to rats since the animals tolerated daily doses as high as 50000 ppm (more than 3000 mg/kg bw/day) without mortality or clinical signs of overt toxicity and without pathological changes other than the rather equivocal salivary gland findings.

B.6.3.2.2 90-day studies in mice

There are only two acceptable studies of which one ([REDACTED] 1991, TOX9552363) was already available for the previous evaluation and is described in the old DAR (1998, ASB2010-10302). A further, rather old study [REDACTED], 1979, TOX9552360) does not comply to modern standards, is now considered not acceptable by the RMS and was deleted from current new assessment. A main deficiency of this study was that hematological and clinical chemistry evaluations were not included.

Below, the new study by [REDACTED] (1995, ASB2012-11453) is described in detail and commented by the RMS.

New subchronic feeding study in mice [REDACTED] 1995)

Reference: IIA, 5.3.2/05

Report: [REDACTED], M. 1995, HR-001: 13-week Subchronic Oral Toxicity Study in Mice.

Laboratory Report No.: [REDACTED] 94-0136

Data owner: Arysta LifeScience

Date: 1995-07-24

not published, ASB2012-12453

Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985;

US. EPA FIFRA Guidelines Subdivision F, 1984

OECD 408 (1981)

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 1994-12-15 to 1995-07-24

Materials and methods

Test material: Glyphosate technical

Identification: HR-001

Description: White crystal

Lot/Batch #: T-941209

Purity: 97.56 %

Stability of test compound: 26/12/1994

Vehicle: Plain diet / none

Test animals:

Species: Mouse

Strain: Crj:CD-1

Source: [REDACTED]

Age: 5 weeks

Sex: Male and female

Weight at dosing: ♂ 27.3-32.7 g; ♀ 22.4-25.8 g

Acclimation period: 9 days

Diet/Food: MF Mash (Oriental Yeast Co., Ltd.)

Water: Filtered and sterilized tap water, *ad libitum*

Housing: 3/cage, sexes separately in stainless steel cages 21.5 x 33.0 x 18.0 cm

Environmental conditions: Temperature: 24 ± 2 °C
Humidity: 55 ± 15 %
Air changes: 15/hour
12 hours light/dark cycle

In life dates: 1995-01-10 to 1995-04-27

Animal assignment and treatment:

The test material was offered on a continuous basis in the basal diet to groups of 24 SPF ICR mice (Crj : CD-1) (12 males + 12 females) for a minimum of 90 days. Dietary concentrations were 0, 5 000, 10 000 and 50 000 ppm.

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level taken from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality: Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations: Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight: Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weights were recorded for all animals before necropsy.

Food consumption and utilisation: Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumption by the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.

Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Group mean chemical intake (mg/kg/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.

Ophthalmoscopic examination: Ophthalmological examinations including observation with a halogen ophthalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13. The following parts of the eye were inspected: Eyeball, cornea, anterior chamber, pupil, iris.

Haematology and clinical chemistry: After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomized under anesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.

The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC).

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.

The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ -glutamyl transpeptidase (GGT), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P).

Urinalysis: At 13 week of treatment, all surviving animals were subjected to urinalysis. Fresh urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was determined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semiquantitatively analyzed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology: Clinical pathology evaluations were also conducted. Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.

The following organs/tissues were taken and preserved: brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibia, femoral joint, lymph nodes, heart, aorta, pharynx, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

Statistics: All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologic parameters, blood chemical parameters, and organ weights).

Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

Results and discussion

Mortality: There were no animals found dead or had to be killed *in extremis* in any groups during the treatment period.

Clinical observations: There were no treatment-related abnormalities in clinical signs in the control and treated groups during the treatment period.

Body weight: In the 50 000 ppm group, mean body weights of males were lower than those of the control from week 2 to the end of the treatment period. Mean body weight of males at week 13 was 91% of that of control. Body weights of females were comparable to the control during the treatment period.

In the groups treated at 10 000 ppm or less, body weights of males and females were comparable to the controls during the treatment period.

Food consumption and test substance intake: In males of the 50 000 ppm group, a significant depression of food consumption was recorded at week 1. Average food consumption of males during the treatment period was 94 % of the control value. Food consumption of females were comparable to the control.

In the groups treated at 10 000 and 5 000 ppm, food consumption of males and females was comparable to that of the controls.

The average daily chemical intakes during the treatment are shown in the following Table B.6.3-15:

Table B.6.3-15: Mean daily glyphosate intake in the study by [REDACTED] (1995, ASB2012-11453)

Dose level (ppm)	Average chemical intake (mg/kg bw / day)	
	Male	Female
5 000	600.2	765.0
10 000	1221	1486
50 000	6295	7435

In the 50 000 ppm group, food efficiency of males and females was lower than that of the controls at almost all measuring points during the treatment. Average food efficiency of males and females was calculated to reach only 79 % and 88 % of the respective control value.

In the groups treated at 10 000 and 5 000 ppm, food efficiency in the treated groups of both sexes was comparable to that in the controls though some significant fluctuations were recorded sporadically.

Ophthalmoscopic examination: There were no ophthalmological abnormalities in the animals of both sexes in the highest dose group and the control group.

Haematology: In the 50 000 ppm group, females showed significant decreases in hematocrit (Ht), hemoglobin concentration (Hb) and erythrocyte count (RBC) by up to 92 % while males showed no significant differences from the control in any parameters.

There were no significant differences in any parameters between the treated groups of 10 000 ppm or less and the control of either sex..

Blood clinical chemistry: The statistically significant changes observed in the treated groups are summarized in the following table (Table B.6.3-16):

Table B.6.3-16: Alterations in clinical chemistry parameters in mice fed glyphosate for 90 days (1995, ASB2012-11453)

Parameter	Sex	Dose group (ppm)		
		5 000	10 000	50 000
Alkaline phosphatase (ALP)	Male	No change	No change	↗ 184*
	Female	No change	No change	↗ 150
Glutamic pyruvic transaminase (GPT)	Female	No change	No change	↘ 69
Creatine phosphokinase (CPK)	Female	↗ 361	No change	↗ 943
Blood urea nitrogen (BUN)	Female	No change	↗ 119	No change
Inorganic phosphorus (P)	Female	No change	No change	↗ 128

* The figures represent the mean percentage of change as compared to the respective control.

In the 50 000 ppm group, males and females showed a significant increase in alkaline phosphatase (ALP). In females, creatine phosphokinase (CPK) and inorganic phosphorus (P) were significantly increased, while a significant decrease in glutamic pyruvic transaminase (GPT) was noted.

In the 10 000 ppm group, females exhibited a significant increase in blood urea nitrogen (BUN). There were no significant change in any parameters in males.

In the 5 000 ppm group, females showed a significant increase in CPK, while there were no significant change in any parameters in males.

Urine analysis: In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

Organ weights: In the 50 000 ppm group, males and females showed significant increases in both absolute and relative weights of the cecum. The absolute weights of the cecum of males and females were 238 % and 187 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 263 % or 195 % in males or females.

In the 10 000 ppm group, absolute and relative weights of the cecum showed increasing tendencies in males and females. The absolute weight of the cecum of males and females were 115 % and 122 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 111 % or 117 % in males or females.

In the 5 000 ppm, there were no significant changes in any organ weights of males and females.

Gross pathology: In the 50 000 ppm group, males and females showed a significant increase in incidence of distention of the cecum (12/12 in males and 10/12 in females, in contrast to 0/12 in males and females of the control group).

In the 10 000 ppm group, distention of the cecum was observed in one female. There were no significant changes in incidence of any macroscopic lesions in males.

In the 5 000 ppm group, there were no treatment-related abnormalities in males and females.

Histopathology: In the 50 000 ppm group, males showed significant increases in incidence of cystitis of the urinary bladder (4/12 as compared to 0/12 in the control group). There were no significant changes in incidence in females. Although significant increases in incidence of distention of the cecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the cecum.

In the 10 000 and 5 000 ppm groups, there were no significant differences in incidence of histopathological lesions from the control in either sex.

Conclusion by the Notifier

Under the experimental conditions of the study, the No Observed Effect Level (NOEL) is considered to be 5000 ppm (equivalent to 600.2 and 765.0 mg/kg bw/day for males and females, respectively).

RMS comments:

The study is considered acceptable. (It was noted that the study director was in fact [REDACTED] as in a 90-day rat study from the same laboratory. [REDACTED] performed the histopathological examinations and was apparently the report writer.)

Because of the only minor effects (slightly higher blood urea nitrogen, slight caecal distention) at the mid dose level of 10000 ppm (equal to 1221 mg/kg bw/day in males) that were not accompanied by any histopathological findings, this dose is considered the NOAEL. Target organs at the very high dose of ca 6300 mg/kg bw/d (50000 ppm) were the caecum and the bladder. Clinical chemistry findings also suggest a weak effect on the liver. This dose level was clearly toxic as additionally proven by effects on body weight gain, food consumption and efficiency and on red blood cell parameters. Thus, the outcome of this study is in line or at least not in contradiction to the previous study by [REDACTED] (1991, TOX9552363) on CD mice in which no effects were observed up to the top dose level of 4500 mg/kg bw/day.

The lower urinary pH in males in all dose groups is due to acidic properties of the test substance and cannot be considered a toxic effect.

In contrast to the publication by [REDACTED] (1992, TOX9551954, reported below), no histological changes of the salivary glands were observed in this study as they had occurred in another strain from dietary concentrations of 6250 ppm (1065 mg/kg bw/day) onwards. Beside possible strain differences, another explanation might be that [REDACTED] (1995, ASB2012-11453) examined the sublingual and submaxillary glands histologically but not the parotis. In the study by [REDACTED] (1991, TOX9552363), histopathological examination of salivary glands was confined to the submaxillary.

Published information

Glyphosate was tested in the 1980ies in U.S. National Toxicology Program (NTP) for oral subchronic toxicity (Chan and Mahler, 1992, TOX9551954). The following paragraph was partly copied from the previous DAR (1998, ASB2010-10302):

Groups of ten B6C3F1 mice per sex and dose were fed glyphosate (supplied by Monsanto, approximately 99 % pure) for 13 weeks at dietary levels of 0, 3125, 6250, 12500, 25000 or 50000 ppm. Clinical pathology investigations were not performed but all animals were necropsied at study termination. Mice of the highest dose group and of the control group were subjected to complete histopathological examination. In addition, salivary glands were

examined microscopically in all dose groups. There were no clinical signs of toxicity. However, one high dose female died from undetermined causes. Body weight gain was depressed at the two upper dose levels in both sexes. From the dose level of 6250 ppm onwards, a dose-related increase in occurrence and severity of cellular alteration of the parotid salivary gland was noted. This change consisted of basophilia of the acinar cells and in more severely affected glands, the cells and acini also appeared enlarged with an associated relative reduction in the number of ducts. A dose of 3125 ppm (ca 507 mg/kg bw/day seems to represent the NOEL for substance-related effects. However, the extent of investigations performed was rather limited.

B.6.3.2.3 Oral 90-day and 1-yr studies in dogs

For the previous EU evaluation, two 90-day studies in dogs had been submitted ([REDACTED] 1991, TOX9650150; [REDACTED] 1985, TOX9551823) that do not comply with modern standards and were assessed now by the RMS as “not acceptable”. The same holds true for a 12-month study by [REDACTED] (1992, TOX9650153). Therefore, these studies were excluded from current re-evaluation of glyphosate, as well as a one-year study by [REDACTED] (1985, Z35385) that was briefly described in the 1998 DAR (ASB2010-10302) but had never been submitted as part of an EU dossier, neither in the 1990ies nor in 2012. It is available in Germany but, without effects up to the highest dose level of 500 mg/kg bw/day, this study would not alter overall assessment.

In contrast, the 12-month study of [REDACTED] (1990, TOX9552384) is still considered acceptable from a today's point of view and is included in the respective table in Volume 1 (2.6.3) and in this chapter. It is reported in detail in the old DAR (1998, ASB2010-10302).

In addition, there is an acceptable study in which the formulation MON0139 (62.49 % IPA salt of glyphosate) had been administered for six months in gelatine capsules to Beagle dogs [REDACTED], 1983, TOX9552361). This study is still included since it was found acceptable upon re-evaluation by RMS. At least, it is suitable to show that this salt proved to be of no higher toxicity in dogs than the acid.

Thus, to an even larger extent than in other fields of toxicological testing of glyphosate, evaluation of its toxicity to dogs is based on new studies that are all reported in detail below.

1st new 90-day study in dogs ([REDACTED] 2007)

Reference:	IIA, 5.3.3/01
Report:	[REDACTED] 2007 Glyphosate Technical: 13-Week Toxicity Study By Oral Route (Capsule) In Beagle Dogs [REDACTED] Laboratory Study No.: 29646 TCC Data owner: Nufarm Date: 2007-07-15 not published, ASB2012-11454
Guidelines:	OECD 409 JMMAF 12 NohSan No. 8147
Deviations:	None
GLP:	Yes

Acceptability: *See RMS comment*

Materials and methods

Test material:

Identification: Glyphosate technical

Description: White crystalline powder

Lot/Batch #: H05H016A

Purity: 95.7 %

Stability of test compound: Stable under storage conditions (< 30 °C), light protected;
Expiry date: 2008-03-25

Vehicle: Empty gelatine capsules, size 12 (Torpac, NY, US)

Test animals:

Species: Dogs

Strain: Beagle

Source: Marshall Farms, North Rose, NY, US

Age: Approx. 6 months

Sex: Male and female

Weight at dosing: ♂ 6.5 – 8.0 kg; ♀ 6.6 – 7.7 kg

Acclimation period: 14 days

Diet/Food: 125 C3 pelleted diet (SAFE, Villemoisson, Epinary-sur-Orge, France), 300 g per day
(Following reduced food consumption among some animals standard tinned dog food was distributed instead or in addition.)

Water: tap water, *ad libitum*

Housing: Individual housing in pens containing wood shavings.

Environmental conditions: Temperature: 20 ± 5 °C

Humidity: 50 ± 20 %

Air changes: 12/hour

12 hours light/dark cycle

In life dates: 2005-06-08 to 2005-09-22

Animal assignment and treatment:

In a 13-week oral toxicity study groups of four Beagle dogs per sex received daily doses of 0, 30, 300 and 1000 mg/kg bw/day glyphosate technical by capsule application. The test item capsules were prepared weekly and delivered daily to the animal room, protected from light. As the test item was put into the capsules, no chemical analysis was performed during the study. The purity, characteristics and identification of the test item were indicated on the certificate of analysis that accompanied the test item.

Mortality: Each animal was checked for mortality or signs of morbidity twice a day during the treatment period, including weekends and public holidays.

Clinical observations: A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before of the beginning of the treatment period and then once a week until the end of the study.

Body weight: The body weight of each animal was recorded twice before group allocation, on the first day of treatment, and then once a week until the end of the study. In addition, the group 4 animals were weighed before final sacrifice on day 75.

Food consumption: The quantity of food consumed was recorded for each animal. Food intake per animal and per day was calculated for 7 days before the beginning of the treatment period and then throughout the study.

Ophthalmoscopic examination: Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry: Haematological and blood chemical and urinalytical investigation were performed on all animals from each test and control group before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3).

Prior to blood sampling the animals were deprived of food for an overnight period of at least 14 hours.

The following parameters were determined: erythrocytes, haemoglobin, MVC, MCHC, MCH, thrombocytes, leucocytes, differential white cell count including morphology, reticulocytes, prothrombin time, activated partial thromboplastin time, sodium, potassium, chloride, calcium, inorganic phosphorous, glucose, urea, creatinine, total bilirubin, total protein, albumin, albumin/globulin ratio, total cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and gamma-glutamyl transferase (GGT).

Urinalysis: Urine samples were collected from all animals of the test and control groups before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3). During urine collection, the animals were deprived of food for an overnight period of at least 14 hours. The following parameters were assessed: appearance, colour, volume, pH, specific gravity, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen, and sediment.

Sacrifice and pathology: On completion of the treatment period (Week 11 or 13), after at least 14 hours fasting, all surviving animals (as well as moribund dogs before) were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thymus, thyroids with parathyroid and uterus.

Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum and femur), brain (at three levels), caecum, colon, duodenum, epididymides, oesophagus, eyes, gall bladder, heart, ileum (with Peyer patches), jejunum, kidneys, larynx, liver, lungs (with bronchi), lymph nodes (mandibular and mesenteric), mammary gland, muscle (skeletal), optic nerve, ovaries, oviducts, pancreas, pituitary gland, prostate, rectum, salivary glands (parotid and submandibular), sciatic nerve, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, ureters, urinary bladder, uterus (horns and cervix) and vagina.

Statistics: Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002, ASB2013-3754), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

Results and discussion

Mortality: Two unscheduled sacrifices (one male and one female) were necessary in animals given 1000 mg/kg bw/day:

One male was sacrificed on Day 61 on humane grounds. Vomiting was seen once in Week 7 (before dosing) and liquid faeces were noted on many occasions in Weeks 8 and 9. Prior to sacrifice, signs of poor clinical condition including thin appearance, dehydration, and pallor of lip mucosa, coldness to the touch, hypothermia (34 to 35 °C) and hypoactivity were observed. These signs were associated with a body weight loss between Weeks 7 and 9 (-34 %) and reduced food consumption from Week 7 (generally only 25 to 50 % of this animal's daily ration was consumed), followed by an absence of food intake on the day before death. Medical care (Smecta® and Lactate Ringer®) was given in order to stop the diarrhoea and rehydrate the animal.

One female was sacrificed on Day 72 for humane reasons. This animal showed liquid or soft faeces on many occasions from Week 4 and dehydration from Week 9. Vomiting was observed once in Week 10. These signs were accompanied by a body weight loss between Weeks 8 and 11 (-22 %) and decreased food consumption from Week 8 (generally only 25 to 50 % of this animal's daily ration was consumed), followed by an absence of food intake on the two days prior to sacrifice. Medical care (Smecta® and lactate Ringer®) was given in many occasions.

Clinical observations: No treatment-related clinical signs were noted in control animals or those given 30 or 300 mg/kg bw/day.

The following treatment-related clinical signs were reported in animals given 1000 mg/kg/day (excluding those killed in extremis, which are discussed separately): liquid or soft faeces on several occasions in all animals, vomiting in 2/3 females on one occasion within 30 minutes or 3 to 5 hours after treatment, thin appearance in 1/3 males and all females, dehydration in 1/3 males and 2/3 females, pallor of ears and mouth in 1/3 females.

Body weight: No relevant differences in the mean body weight gain were noted between controls and animals given 30 or 300 mg/kg bw/day during the treatment period.

Due to numerous individual body weight losses recorded from Week 4 in males and from Week 1 in females, a marked lower mean body weight was noted in animals given 1000 mg/kg/day at termination.

At the end of the treatment period this resulted in only a slight mean body weight gain in males (+4 % vs. +31 % in controls) and a mean body weight loss in females (-7 % vs. +14 % in controls) when compared to their body weight on Day 1. This effect on body weight was considered treatment-related (see Table B.6.3-17).

Table B.6.3-17: Group mean weekly bodyweights and standard deviations (sd)

Time point	Mean bodyweight and body weight change (kg)						
	Day 1	Week 5	Week 9	Week 11	Change week 1-11	Week 13	Change week 1-13
Dose (mg/kg bw/day)	Males						
0	7.4	9.0	9.5	9.7	+2.3	10.4	+3.0
30	7.2	8.5	8.9	9.1	+1.9	9.5	+2.3
300	7.3	8.5	9.0	9.2	+1.9	9.7	+2.4
1000	7.3	8.3	7.7*	7.6*	+0.3	na	na
	Females						
0	7.3	7.8	8.2	8.2	+1.0	8.8	+1.5
30	7.3	8.3	8.7	8.7	+1.6	9.2	+1.9
300	7.4	8.2	8.6	8.6	+1.3	9.2	+1.8
1000	7.2	7.0	6.9	6.9	-0.5	Na	na

* Statistically significant from controls ($p < 0.05$).

na not applicable due to premature sacrifice

Food consumption: The food consumption was not affected by the test article treatment in animals given 30 and 300 mg/kg bw/day. Reduced food consumption, varying from 25 to 75 % of the amount given, was observed on many occasions in animals given 1000 mg/kg bw/day. From Day 62, when tinned dog food was distributed instead of pelleted diet, all animals consumed their full daily ration.

Ophthalmoscopic examination: There were no ophthalmological findings at the end of the treatment period.

Haematology and clinical chemistry: The laboratory investigations of the moribund sacrificed male showed the following changes among haematological and blood biochemical parameters when compared to pre-treatment values:

increase in leucocyte count mainly due to an increase in the neutrophil count,

increase in haemoglobin level, erythrocyte count and packed cell volume,

decrease in platelet count,

decrease in sodium and chloride levels, as well as an increase in potassium and inorganic phosphorus levels,

increase in glucose, protein, albumin, cholesterol, triglycerides, urea and creatinine levels.

Some of the abnormalities found in the laboratory investigations (such as the increase in red blood cell parameters and in protein and albumin levels) were indicative of haemoconcentration, which was probably secondary to the dehydration caused by the diarrhoea.

The laboratory investigations performed before sacrifice of the moribund female dog showed the following changes among the blood biochemical parameters when compared to pre-treatment values:

decrease in sodium, potassium, chloride and inorganic phosphorus levels,

decrease in urea, protein and albumin levels and increase in total bilirubin level and alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities.

The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration).

In the following the results of the laboratory investigations of the surviving animals are summarised.

Haematology: When compared to both pre-dose and control values, no biologically relevant differences were noted in surviving animals of the test item groups in Weeks 7 and 11/13.

Blood chemistry: When compared to control values in Week 13, the following test-substance related differences were noted in animals given 1000 mg/kg bw/day in Week 11 (see Table B.6.3-18): higher alanine aminotransferase (ALAT) activity in 2/3 males and 1/3 females, lower alkaline phosphatase (ALP) activity in 3/3 females, lower protein and albumin levels in 3/3 females.

Other changes were not attributed to the test item-treatment.

Table B.6.3-18: Group mean blood chemical values and standard deviations (sd) in Week 11/13

Dose (mg/kg bw/d)		ALAT (IU/L)	ALP (IU/L)	Total protein (g/L)	Albumin (g/L)
Males					
0	mean	31	–	–	–
(Week 13)	sd	4.8	–	–	–
30	mean	34	–	–	–
(Week 13)	sd	5.32	–	–	–
300	mean	30	–	–	–
(Week 13)	sd	8.9	–	–	–
1000	mean	91	–	–	–
(Week 11)	sd	42.5	–	–	–
Females					
0	mean	29	388	61	35
(Week 13)	sd	6.0	168.0	2.1	1.6
30	mean	31	281	62	34
(Week 13)	sd	10.4	91.5	2.1	1.0
300	mean	29	332	59	35
(Week 13)	sd	4.1	142.6	2.5	0.6
1000	mean	122	321	55	30
(Week 11)	sd	163.9	322.0	5.5	2.5

– – no relevant changes

Urine analysis: When compared to both pre-dose and control values, the following findings were noted at 1000 mg/kg bw/day in Week 11:

decrease in mean specific gravity in 1/3 males and 3/3 females,

increase in mean urinary volume accompanied by less marked colour of urine in 3/3 females.

As these changes were only noted at the highest dose-level, they were attributed to the test item treatment.

Organ weights: Treatment-related, statistically significant effects (reduction) were restricted to the prostate.

Gross pathology: Macroscopic pathological examination of the male that was killed moribund demonstrated a reddish mucosa of the colon and rectum appeared, enlarged adrenal glands and thyroids, and reduced size of the spleen and thymus.

In the high-dose female that was killed moribund, the oesophagus, jejunum and ileum presented many greyish/white areas and the colon mucosa showed reddish/purplish foci. The gall bladder was dilated with blackish deposits and the liver was yellowish, enlarged and firm. The kidneys were pale.

All the macroscopic changes noted in surviving animals at termination were considered to be normal variations, when compared to background data, which may be seen in untreated

beagle dogs of this age, except for changes in the uterus (reduced in size) for females given 1000 mg/kg bw/day.

Histopathology: The major histopathological findings in the male dog sacrificed moribund were bilateral hyaline degeneration of the cortical tubules in the kidneys with pigment deposits, diffuse acute inflammation in the liver with pigment deposits, acute inflammation of the lamina propria of the oesophagus, bilateral hypertrophy of cortex of the adrenals, diffuse lymphoid atrophy in the spleen, acute inflammation in the lungs with alveolar spaces containing blood and increased number of adipocytes in the sternum.

The bilateral hyaline degeneration of the cortical tubules in the kidneys was considered to be test item treatment-related. However, it is not possible to determine if this lesion, which was associated with increase in urea and creatinine levels, was directly due to the test item action or the result of the dehydration caused by a severe intestinal irritation. The inflammation noted in the liver, oesophagus and lungs was considered to be test item related and was associated with change in leucocyte count. The increased number of adipocytes in the sternum seen also in the schedule killed animals was considered treatment-related. The abnormalities reported in blood electrolyte levels, glucose, triglycerides and cholesterol levels were not directly attributed to the test item treatment but were considered to be secondary to the poor clinical condition of the animal (diarrhoea, dehydration, changes in the kidneys). The modifications reported in spleen and adrenal glands were not attributed to the test item treatment, as they were non-specific changes that could be found in treated animals housed in laboratories.

At microscopic level, the major findings in the sacrificed female were bilateral vacuolation of the cortical tubules in the kidneys, macrovesicular vacuolation in the liver, diffuse hypoplasia of langerhans islet in the pancreas, severe atrophy of cortex of the thymus, increased number of adipocytes in the sternum and uterine atrophy.

The liver histopathological modification was considered to have resulted from the test item treatment and was correlated with changes in the blood biochemical parameters (i.e. urea, protein, albumin and bilirubin levels as well as liver enzyme activities). The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration). The uterine atrophy and increased number of adipocytes in the sternum, seen also in the schedule killed top dose animals, were considered treatment-related.

The atrophy noted in the thymus is a non specific change that could be found in laboratory housed animals; therefore a relationship to the test treatment was excluded. The other lesions noted (i.e. in the kidneys and pancreas) can be spontaneously observed in untreated beagle dogs of this age and sex. Therefore a relationship to the test treatment was considered unlikely.

No test-substance related histopathological changes were observed in animals of both sexes at and below 300 mg/kg bw/day.

Treatment-related changes observed in surviving animals given 1,000 mg/kg bw/day consisted of increased number of adipocytes in the sternum of 2/3 males and 3/3 females, prostate atrophy in 2/3 males and uterine atrophy in 2/3 females.

These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused by the test item.

All the other microscopic findings observed in the organs of both male and female animals of the high-dose group were judged to be unrelated to treatment or normal background findings.

Conclusion by the Notifiers

Under the experimental conditions of the study and taking into account the slight effects on organ weights at the mid dose-level, the No Observed Adverse Effect Level (NOAEL) is considered to be 300 mg/kg bw/day.

RMS comments:

The study is considered acceptable and the NOAEL is agreed. At the top dose level, the MTD was clearly exceeded. It was noticed that high dose effects of glyphosate administration in this study were particularly severe, much more pronounced and rather different from what was seen in other dog studies or other species. Thus, because of the clinical signs and pathological changes, its results do not fit into the toxicity profile of glyphosate as it was established in the majority of studies. In the study by [REDACTED] (1990, TOX9552384) that is described in detail in the original DAR (1998, ASB2010-10302), the same high dose of 1000 mg/kg bw/day was administered also in capsules 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. In any case, it should be taken into consideration that this dose level is by 2000 times higher than the proposed ADI.

2nd new 90-day study in dogs ([REDACTED] 1999)

Reference: IIA, 5.3.3/02

Report: [REDACTED] 1999 Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs
AND
Test compound stability in experimental diet (dog feed)

[REDACTED]
Data owner: Feinchemie

Study No.: 1816 AND 1817-R.FST

Date: 1999-04-17 AND 1997-02-21

not published, ASB2012-11455

Guidelines: OECD 409

Deviations: Several organ weights missing: epididymis, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled (gross, lesions. Spinal cord, eyes with optic nerve, trachea and mammary gland.

GLP: Yes

Acceptability: See RMS comment

Materials and methods

Test material:

Identification: Glyphosate technical

Description: Crystalline solid

Lot/Batch #: 01.12.1997 & 01.06.97

Purity: > 95 %

Stability of test compound: Expiry dates: 2000-06-01 and 2000-12-01

Vehicle: Plain diet

Test animals:

Species:	Dogs
Strain:	Beagle
Source:	
Age:	6 - 8 months
Sex:	Male and female
Weight at dosing:	♂ 10.0 – 12.2 kg; ♀ 8.8 – 11.0 kg
Acclimation period:	6 days
Diet/Food:	Nutripet Pet meal (Tetragon Chemie Pvt.Ltd., Bangalore, India), was offered daily for one hour <i>ad libitum</i>
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, <i>ad libitum</i>
Housing:	Individual housing in floor pens.
Environmental conditions:	Temperature: 23 - 29 °C Humidity: 40 - 70 % Air changes: no data Natural daylight plus fluorescent light from 9 am to 5 pm

In life dates: 1998-03-18 to 1998-06-26

Animal assignment and treatment:

In a 90 day feeding study groups of four Beagle dogs per sex received daily doses of 0, 200, 2000 and 10,000 ppm Glyphosate technical in the diet (corresponding to 5.3, 53.5 and 252.6 mg/kg bw/day).

Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending. This pre-mix was then added to larger amount of basal diet and blended for 20 minutes. The feed was fortified with test compound at weekly intervals.

The stability of the test compound was examined in an additional study (No. 1817-R.FST). The homogeneity of the test material in diet was determined at start of the study. Three samples from the food fortified with the test compound were taken and analyzed.

Mortality: Each animal was checked for mortality or signs of morbidity daily during the treatment period.

Clinical observations: Each animal was daily checked for signs of toxicity. A more detailed veterinary investigation was performed before start of exposure, monthly throughout the study and before termination.

Body weight: The body weight of each animal was recorded before allocation and start of treatment, weekly throughout the study and before termination.

Food consumption: The quantity of food consumed was recorded for each animal on a weekly basis.

Ophthalmoscopic examination: Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry: Haematological and blood chemical investigation were performed on all animals from each test and control group before the beginning of the treatment period, after 45 days of exposure and at termination from animals fasted since the last feeding.

The following parameters were determined: erythrocytes (RBC), haemoglobin (HB), hematocrit (HCT), MVC, MCHC, MCH, leucocytes (WBC), differential white cell count (Neut, Lymph, Eos, Mono, Retic), clotting time, glucose, urea, total protein, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatinine, total bilirubin, albumin, calcium, inorganic phosphorous, total cholesterol, triglycerides, chloride, sodium, potassium.

Urinalysis: Urine was collected from all animals at termination during autopsy. Urinalysis was performed for control and high-dose group animals.

The following parameters were determined: pH, specific gravity, leucocytes, proteins, glucose, ketones, blood, and urobilinogen.

Sacrifice and pathology: On completion of the treatment period, after an overnight fasting, all surviving animals were killed and subjected to a gross pathological examination. The moribund animals were sacrificed in the same way. Any macroscopic findings were recorded. The following organ weights were determined: adrenals, kidneys, liver (with gall bladder), testes and thyroids with parathyroids.

Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum), brain, caecum, colon, duodenum, gall bladder, gonads, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric), oesophagus, pancreas, pituitary gland, rectum, salivary glands, sciatic nerve, spleen, stomach, thymus, thyroids with parathyroids, urinary bladder, uterus. These tissues (plus parathyroids) were microscopically investigated for all animals of the control and high dose group.

Statistics: Body weights, net body weight gain, food intake, laboratory investigations (haematology and clinical chemistry values of days 0, 45 and 90), organ weights data and organ weight ratios were compared by Bartlett's test for homogeneity of intra group Variances. When the Variances proved to be heterogeneous, the data were transformed using appropriate transformation.

The data with homogeneous intra group variances were subjected to one-way analysis of variance. Following ANOVA, when F was found to be significant, Dunnett's pair wise comparison of means of treated groups with control mean was done individually. Following a significant difference of a test group with the control group, the Dose Response correlation was estimated including the control and all treated groups and tested by 't'-test. All analyses and comparisons are evaluated at 5 % probability level.

Results and discussion

Mortality: All animals survived until scheduled necropsy.

Clinical observations: No clinical signs of toxicity were observed.

Body weight: Body weights remain essentially unaffected from treatment. A slight initial depression of body weight gain in the high dose male and female groups might be concluded (and would be in accordance with the food consumption).

Table B.6.3-19: Group mean body weights

	Body weight [kg]													
week	-1	1	2	3	4	5	6	7	8	9	10	11	12	13
Test item [ppm]	Males													
0	11.1	11.7	12.2	12.3	12.5	12.7	12.9	13.2	13.3	13.4	13.5	13.6	13.7	13.8
200	11.2	11.6	12.1	12.3	12.8	12.7	13.2	13.5	13.5	13.5	13.6	13.7	13.6	13.5
2000	11.1	11.7	12.1	12.0	12.6	12.7	12.9	13.2	13.4	13.4	13.3	13.3	13.2	13.2
10000	11.1	11.4	11.2	11.5	12.4	12.4	12.7	12.8	13.0	13.3	13.3	13.5	13.3	13.6
Test item [ppm]	Females													
0	10.1	10.4	10.9	10.7	11.0	11.0	11.2	11.4	11.7	11.9	11.7	12.0	11.7	11.8
200	10.2	10.7	11.1	10.9	11.3	11.4	11.5	11.7	11.8	11.5	11.6	11.6	11.6	11.4
2000	9.8	10.2	10.3	10.2	10.8	10.9	11.0	11.2	11.4	11.4	11.4	11.5	11.6	11.5
10000	10.0	9.9	10.1	10.1	10.6	10.6	10.7	10.9	11.2	11.3	11.3	11.3	11.3	11.2

Food consumption: The food intake of the high dose group (10,000 ppm) was significantly lower during the second week of treatment only. Except this finding the food consumption of all the treatment groups were comparable to the control group during the study period.

Table B.6.3-20: Average weekly food intake

	Food consumption [g/animal/day]												
week	1	2	3	4	5	6	7	8	9	10	11	12	13
Test item [ppm]	Males												
0	254	336	342	336	320	346	345	325	312	332	356	368	369
200	287	354	366	376	326	346	350	342	325	343	318	347	312
2000	305	373	406	347	334	363	358	366	339	321	330	329	339
10000	262	177*	380	368	342	332	330	324	328	303	341	333	330
Test item [ppm]	Females												
0	247	263	278	302	295	298	283	297	275	290	295	292	285
200	285	332	324	352	323	321	292	294	294	325	317	290	271
2000	212	306	338	309	298	286	290	298	284	278	285	303	283
10000	212	166*	348	327	303	261	288	294	298	262	268	262	256

The calculated mean daily test substance intake is summarised in Table B.6.3-21 below.

Table B.6.3-21: Group mean compound intake levels

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	200	5.2	5.4	5.3
3 (mid)	2000	54.2	52.8	53.5
4 (high)	10000	252.4	252.7	252.6

* based on actual food intake and body weight data

Ophthalmoscopic examination: There were no ophthalmological findings at the beginning and at the end of the treatment period.

Haematology and clinical chemistry: A significant increase in clotting time and GGT-activity was observed in both sexes at the 45-day interim bleed; however, in absence of any corresponding changes at terminal bleed or any histopathological correlate in the liver, this observation is considered to rather reflect a systemic error during determination than a real effect of the test item.

Table B.6.3-22: Summary of results for clotting time

	Clotting time [s]							
[ppm]	0	200	2'000	10'000	0	200	2'000	10'000
	males				Females			
Pre-exposure bleed	145	150	147	144	154	162	149	131
45 day interim bleed	131	153*	172*	183*	141	161*	173*	182*
90 d final bleed	134	134	136	139	142	142	134	138

Table B.6.3-23: Summary of results for gamma-GT activity

	GGT [U/L]							
[ppm]	0	200	2'000	10'000	0	200	2'000	10'000
	males				Females			
Pre-exposure bleed	9	10	8	7	9	7	7	11
45 day interim bleed	13	13	16	19*	14	14	14	21*
90 d final bleed	11	12	16	18	17	16	16	29

Total bilirubin was higher; however, in absence of a histopathological correlate on the liver, the effect was not considered adverse.

Table B.6.3-24: Summary of results for total bilirubin

	Total bilirubin [μmol/L]							
[ppm]	0	200	2'000	10'000	0	200	2'000	10'000
	Males				females			
Pre-exposure bleed	3.71	3.99	3.71	3.14	3.67	3.51	3.96	4.02
45 day interim bleed	5.25	5.10	5.93	5.97	5.22	5.23	6.49*	6.54*
90 d final bleed	4.21	5.65*	5.95*	6.21*	4.00	6.57*	7.08*	7.18*

Urinalysis: All parameters were in the normal range and comparable between control and treated animals.

Organ weights: No treatment-related effects were observed.

Necropsy: No treatment-related gross pathological effects were observed.

Histopathology: There were a few incidental findings with equal distribution across control and treated groups – no relation to treatment was observed.

Conclusion by the Notifiers

Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) of glyphosate Technical in Beagle dogs is considered to be 10,000 ppm (252.6 mg/kg bw/day).

RMS comments:

The study is considered acceptable. It is agreed to consider the highest dose the NOAEL because the minor effects were indeed not adverse. The lower body weight gain at the beginning of treatment is very probably a result of impaired food consumption. Lower food intake might be due to a palatability problem or might simply result from the need of the animals to adapt to a diet with a new and perhaps strange taste. The higher bilirubin levels might be due to treatment but were not accompanied by any pathological change.

It was noted that the highest dose chosen, as compared to other studies with dietary administration to dogs, was rather low.

3d new 90-day study in dogs ([REDACTED] 1996)

Reference: IIA, 5.3.3/03

Report: [REDACTED] 1996 HR-001: 13-week Subchronic Oral Toxicity Study in Dogs.

[REDACTED]
Laboratory Report No.: IET 94-0158

Data owner: Arysta LifeScience

Date: 1996-09-05

not published, ASB2012-11456

Guidelines: Japan-MAFF Guidelines 59 NohSan No.4200, 1985,
U.S. EPA 2012-11456)FIFRA Guidelines Subdivision F, 1984
OECD 409 (1981)

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Materials and methods

Test material: Glyphosate technical

Identification: HR-001

Description: White crystal

Lot/Batch #: T-940308

Purity: 94.61 %

Stability of test compound: Not mentioned in the report

Vehicle: Plain diet / none

Test animals:

Species:	Dog
Strain:	Beagle
Source:	
Age:	♂ 5 months; ♀ 6 months
Sex:	Male and female
Weight at dosing:	♂ 27.3-32.7 g; ♀ 22.4-25.8 g
Acclimation period:	♂ 21 days; ♀ 50 days
Diet/Food:	Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day
Water:	Filtered and sterilized tap water, <i>ad libitum</i>
Housing:	Individually in stainless steel cages 83.5 x 90.0 x 80.0 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle

In life dates: 1995-09-20 to 1996-02-08

Animal assignment and treatment:

The test material was offered on a continuous basis in the basal diet to groups of 4 males and 4 females Beagle dogs for a minimum of 90 days. Dietary concentrations were 0, 1 600, 8 000 and 40000 ppm.

Homogeneity of the test substance in diet was ascertained for all dose levels using the samples taken from the top, middle and bottom portions of the mixer at the first diet preparation (before initiation of the study). The coefficient of variation of the concentrations of technical glyphosate was 2.3 % or less for all test diets and confirmed that the test substance was mixed in the basal diet at good homogeneity.

Concentrations of technical glyphosate in test diets were monitored for all batches of test diets of all dose levels during the study. The overall mean concentrations found in test diets were within a range of 94–101 % to the nominal levels and confirmed that the test substance was mixed in the test diets at acceptable concentrations.

Mortality: Mortality was expressed weekly as a ratio of the cumulative number of animals found dead or killed in extremis to the effective number of animals per dose group.

Clinical observations: Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight: Body weights of all animals were recorded at initiation of treatment and weekly during the study. In addition, final body weight of each animal was measured before necropsy.

Food consumption and utilisation: Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:

Chemical intake (in mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.

Ophthalmoscopic examination: Prior to initiation of treatment and at week 13, all animals were subjected to ophthalmological examinations with a direct ophthalmoscope.

The following parameters were determined: Eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, and fundus.

Haematology and clinical chemistry: Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to haematological examinations. Blood samples were withdrawn with heparinised syringes from the cephalic vein of the animals following overnight starvation. A part of each sample was transferred to a cup of treated with EDTA and subjected to the haematological examination.

The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC).

Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to biochemical examinations. Plasma from heparinised blood sample from haematological tests were used.

The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ -glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P).

Urinalysis: Prior to initiation of treatment and at week 13 of treatment, all animals were subjected to urinalysis.

Volume and sediments were determined on urine samples collected for 24 hours using trays.

The other parameters were determined on fresh urine samples.

The following parameters were determined: specific gravity, pH, protein, glucose, ketones, accult blood, urobilinogen, bilirubin, appearance urine volume, urinary sediments.

Sacrifice and pathology: All animals were subjected to a complete necropsy and all gross findings were recorded. After 13 weeks of treatment, all animals were anesthetized and euthanized by exsanguinations from the carotid artery before necropsy. At necropsy the organs and tissues except eyes were removed and preserved in neutral-buffered 10 % formalin. The eyes were fixed in a phosphate-buffered mixed solution of formalin and glutaraldehyde for about 3 days and transferred to neutral-buffered 10 % formalin.

Weights of the following organs were recorded for all animals and the ratios to the final body weight were calculated: brain, heart, adrenals, thyroids with parathyroids, liver, ovaries, kidneys, prostate, spleen.

The following organs and tissues from all animals were histopathologically examined: brain, spinal cord, peripheral nerve, pituitary, thyroids with parathyroids, thymus, adrenals, tonsil, spleen, bone with marrow, lymph nodes, hearth, aorta, tongue, pharynx, buccal mucosa of

oral cavity, salivary glands, esophagus, stomach, liver, gallbladder, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, epididymides, penis, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland, all gross lesions

Statistics: All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologic parameters, blood chemical parameters, and organ weights).

Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

Results and discussion

Mortality: There were no animals found dead or killed *in extremis* in any groups during the treatment period.

Clinical observations: Statistically significant differences in incidence of clinical signs were not observed between the control and treated groups in either sex.

Body weight: Statistically significant differences in body weights were not observed between the control and treated groups in either sex throughout the treatment.

Food consumption and test substance intake: There were no significant changes in food consumption and chemical intake in either sex of the treated groups.

The overall group mean chemical intakes (mg/kg bw/day) over the whole treatment period were calculated from food consumption, body weights, and the nominal dose levels. The results are shown in the table below:

Table B.6.3-25: Summary of compound intake

Dose level (ppm)	Overall group mean chemical intake (mg/kg bw/day)	
	Male	Female
1 600	39.7	39.8
8 000	198	201
40 000	1015	1014

Ophthalmoscopic examination: No ocular changes were detected in any dose groups of both sexes.

Haematology: Statistically significant changes in haematology parameters were observed in the treated groups as shown in the following table:

Table B.6.3-26: Results of haematological examinations

		Dose group (ppm)								
		1 600			8 000			40 000		
Week of treatment		0	7	13	0	7	13	0	7	13
Erythrocyte count (RBC)	Male	-	-	↑ (112)	-	-	↑ (115)	-	-	-
Mean corpuscular volume (MCV)	Male	↘ (89)	-	-	-	-	-	-	-	-
Mean corpuscular hemoglobin concentration (MCHC)	Female	-	-	-	-	-	-	↓ (96)	-	↘ (97)
Lymphocytes (Lym)	Female	↘	-	-	-	-	-	-	-	-

RMS comments:

The study is considered acceptable. The highest dose level of 40 000 ppm is considered the NOAEL because there were no adverse effects of treatment observed. The decrease in urine pH in some high dose females is most likely due to the acidic properties of the test substances and was measured in other toxicological studies before and after, too. Occurring in isolation, without concomitant signs of renal or bladder toxicity, this is not considered an adverse finding.

4th new 90-day study in dogs [REDACTED] 1996)

Reference: IIA, 5.3.3/04
Report: [REDACTED] 1996
 First Revision To Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs
 [REDACTED]
 Cheshire, UK
 Data owner: Syngenta
 Report No.: [REDACTED]/P/1802
 Date: 1996-11-14
 not published, TOX2000-1991
Guidelines: OECD 409 (1998): OPPTS 870.3150 (1998): 2001/59/EC B.27 (2001)
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Materials and methods

Test Material: Glyphosate acid
 Description: Technical, white solid (passed through a 75 µm mesh)
 Lot/Batch number: D4490/1, P18
 Purity: 99.1 % w/w a.i.
 CAS#: Not reported
 Stability of test compound: Not reported
 Vehicle: Glyphosate acid was administered in diet.
 Test Animals:
 Species: Dog
 Strain: Beagle
 Age/weight at dosing: 22 - 26 weeks
 Source: [REDACTED]
 Housing: Individually in indoor pens, with a floor area of 345 x 115 cm. Each pen consisted of an exercise area and separate sleeping quarters with a heated floor.
 Acclimatisation period: 4 – 5 weeks
 Diet: Laboratory Diet A (Special Diet Services Ltd., Witham, Essex, UK) *ad libitum*
 Water: Mains water *ad libitum*
 Environmental conditions: Temperature: 19 - 22 °C

Humidity: Not reported

Air changes: Approximately 12 changes / hour

Photoperiod: 11 hours light / 13 hours dark

In-life dates: Start: 12 August 1986

End: 19 November 1986

Animal assignment: The study consisted of one control and three treatment groups each containing 4 male and 4 female dogs. The randomisation procedure employed ensured the even distribution of animals across replicates (randomised blocks) and treatment groups, by bodyweight, placing litter mates in different treatment groups. The sexes were randomised separately.

Male dogs received 400 g and females 350 g of the appropriate diet, in the morning between 9 am and 12 noon each day. During the pre-study period, the food was removed 2-5 hours after presentation in an attempt to ensure that the dogs ate the diet rapidly. Several batches of test diets were prepared so that no one batch was fed for longer than 5 weeks.

The clinical condition and body weights of the dogs were monitored during the study, as was their biochemical and haematological status. At the end of the study the dogs were subjected to an examination *post mortem*. The major organs were fixed, processed and examined.

Diet preparation and analysis: All experimental diets were based on expanded, ground Laboratory Diet A.

The glyphosate acid concentration was determined for each occasion diet was mixed. The homogeneity of diets containing glyphosate acid was established by analysis of aliquots of diet taken from each mix of the low and high dose diet on the first occasion on which diets were prepared. The stability of the low and high dose diets was determined over a 39 day period on one mix from the first occasion on which diets were prepared.

Observations: A detailed clinical examination, which included cardiac and pulmonary auscultation was made on all dogs pre-experimentally and in week 13. In the treatment period, the dogs were observed at least twice during the working day for gross clinical and behavioural abnormalities.

A daily record of faecal consistency was made during the pre-experimental and dosing periods.

Body weight: All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals, until termination.

Food consumption: Food residues were recorded daily and were then discarded. These measurements were made usually 4 hours (between 2-5 hours) after presentation of the diet during the pre-experimental period and approximately 24 hours after presentation of the diet during the dosing period.

Ophthalmoscopic examination: The eyes of all dogs were examined by indirect ophthalmoscopy pre-experimentally and in week 13.

Haematology: Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters measured:

Haemoglobin	mean cell haemoglobin concentration
Haematocrit	platelet count
red blood cell count	total white cell count
mean cell volume	differential white cell count
mean cell haemoglobin	blood cell morphology
kaolin-cephalin time	prothrombin time

Bone marrow smears were taken from a femur of all dogs at necropsy, air dried, fixed in absolute methanol and stored but not examined.

Clinical chemistry: Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters assessed:.

Urea	alkaline phosphatase activity
Glucose	aspartate aminotransferase activity
Albumin	alanine aminotransferase activity
total protein	gamma-glutamyl transferase activity
Cholesterol	calcium
Triglycerides	sodium
creatinine kinase activity	potassium

Urinalysis: Urine was collected by catheterisation from all dogs, once pre-experimentally and in week 13. Microscopic examination of the centrifuged deposits, from all dogs, was made pre-experimentally and in week 13 on the samples taken for biochemical analysis.

Urobilinogen	glucose
specific gravity	ketones
pH	protein
Bilirubin	blood

Macroscopic examination: At the end of the 90 day dosing period, all animals were killed and examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands	ovaries
Brain	liver
Epididymides	testes
Kidneys	thyroid glands (with parathyroids)

The left and right components of paired organs were weighed separately.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

gross lesions including masses	oesophagus
--------------------------------	------------

adrenal gland	ovary
aorta	pancreas
brain	pituitary gland
bone and bone marrow (rib)	prostate gland
caecum	rectum
colon	salivary gland
duodenum	spinal cord (lumbar)
gall bladder	skin
epididymis	spleen
eyes	sternum
femur (including stifle joint)	stomach
heart	testis
ileum	thymus
jejunum	thyroid/parathyroid gland
kidney	trachea
liver	urinary bladder
lung	uterus
lymph node - prescapular	voluntary muscle
lymph node - mesenteric	cervix
mammary gland (females only)	nerve - sciatic

Microscopic examination: All processed tissues were examined by light microscopy.

Statistics: Body weight gains from the start of the study to each week and final body weights were considered by analysis of variance, separately for males and females.

Haematology, blood and urine biochemistry data were considered, at each sampling time after the start of the study, by analysis of co-variance on pre-experimental values. Male and female data were analysed together and the results examined to determine whether differences between control and treated groups were consistent between sexes.

Organ weights at termination were considered by analysis of variance and analysis of co-variance on the last measured body weight, separately for males and females. Left and right components of paired organs were considered separately and combined to investigate for any differential effects.

All analyses allowed for the replicate design of the study and were carried out using *SAS* (1982). Unbiased estimates of the treatment group means were provided by least square means (LSMEANS option in *SAS*). Each treatment group was compared to the control group mean using a two-sided Student's t-test, based on the error mean square from the appropriate analysis. Where male and female data were analysed together, these comparisons were made separately.

All data were checked for atypical values and where such values were detected the analyses were repeated omitting these values to determine their influence on the conclusions.

Results and discussion

Concentration analysis results: The achieved dietary concentrations of glyphosate acid were all within ± 9 % of the target concentrations.

Homogeneity results: The homogeneity was considered to be satisfactory with all the mean values from the analysis at the different sampling points being within 6 % of the overall mean.

Stability results: Over a period of 39 days, no significant change was seen in the chemical stability at 2000 and 50000 ppm glyphosate acid.

Mortality: There were no mortalities.

Clinical observations: The clinical observations noted were of a minor nature, often seen in studies of this duration using this strain of dog, and are considered to be unrelated to treatment with glyphosate acid.

Body weight and weight gain: Body weight gain of males given 50000 ppm glyphosate acid showed a slight depression throughout the study, but the differences were not statistically significant.

Females given 50000 ppm glyphosate acid showed slightly reduced bodyweight gains throughout the study and these were occasionally statistically significantly different from the controls.

There was no effect on growth in dogs given 2000 or 10000 ppm glyphosate acid.

Table B.6.3-28: Intergroup comparison of body weight gain (g) (selected timepoints)

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
week	0	2000	10000	50000	0	2000	10000	50000
Initial wt	10.97	10.60	11.00	10.90	9.70	9.40	9.47	9.47
4	1.00	1.13	1.07	0.65	0.64	0.75	0.85	0.38*
9	2.07	1.92	2.07	1.65	1.31	1.42	1.52	0.97*
Final wt	13.03	13.00	13.37	12.50	11.31	11.13	11.40	10.95

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

Food consumption and utilisation: All dogs ate all the diet presented during the dosing period. The dose received (in mg glyphosate acid/kg/day) was similar for both males and females. During the study, there was the expected decrease in the dose received, due to the increasing weight of the dogs.

One dog fed 10000 ppm glyphosate acid was given cubed diet for two days in week 5 to prevent it scooping up powdered diet and thereby allowing healing to a wound in its front paw. No glyphosate acid was received by this dog on these two days.

Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg body weight. Mean values are shown below:

Table B.6.3-29: Mean Dose Received (mg/kg bw/day)

Glyphosate acid (ppm)	2000	10000	50000
Males	68	323	1680
Females	68	334	1750

Ophthalmoscopic examination: There were no treatment-related ophthalmological findings.

Haematology: There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry: Male dogs fed 50000 ppm glyphosate acid showed slightly reduced plasma albumin and total protein concentrations, possibly representing the start of an expected effect of feeding an inert substance at a sufficiently high level to reduce the intake of nutrients. Plasma calcium levels were also minimally reduced in these animals, possibly a result of calcium sequestration which occurs with compounds structurally-related to glyphosate acid.

Female dogs given 50000 ppm glyphosate acid had slightly elevated plasma alkaline phosphatase activities throughout the study.

There were no treatment-related changes in dogs fed 2000 or 10000 ppm glyphosate acid. There were other isolated instances where results were statistically significantly different from control, but these were considered to be unrelated to treatment.

Table B.6.3-30: Intergroup comparison of clinical chemistry – selected parameters, selected weeks

		Dose Level of glyphosate acid (ppm)							
		Males				Females			
Parameter	Wk	0	2000	10000	50000	0	2000	10000	50000
Albumin	4	3.70	3.70	3.73	3.43*	3.76	3.65	3.89	3.51*
	8	3.77	3.74	3.69	3.53*	3.72	3.71	3.92	3.63
	13	3.92	3.97	3.77	3.66**	3.84	3.70	3.94	3.78
Total protein	4	5.57	5.42	5.34	5.14**	5.36	5.40	5.42	5.22
	8	5.44	5.49	5.32	5.22*	5.32	5.30	5.52*	5.19
	13	5.60	5.70	5.45	5.38	5.39	5.34	5.65*	5.30
Calcium	4	11.2	11.2	11.1	10.5**	10.9	11.1	11.2	10.7
	8	11.2	11.1	10.9*	10.8**	10.9	11.0	11.2*	10.9
	13	10.7	10.5	10.8	10.0**	10.4	10.3	10.6	10.4
plasma alkaline phosphatase	4	182	190	188	193	176	181	182	220**
	8	155	168	164	177	152	155	155	181*
	13	149	165	160	161	140	143	145	166*

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)
 ** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided) Wk – week number

Urinalysis: There were no differences in urine clinical chemistry parameters, nor in urinary sediment examinations, which were considered to be related to treatment.

Organ weights: Kidney weights of males given 10000 or 50000 ppm glyphosate acid were slightly increased above control values, but the increase was not proportional to dose. There was also a small increase in liver weight at these dose levels, but in male dogs only.

Table B.6.3-31: Intergroup comparison of liver weight (g) in male dogs (adjusted for body weight)

0 ppm	2000 ppm	10000 ppm	50000 ppm
385	409	427*	436**

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

Thyroid weights, adjusted for bodyweight, of females given 2000 or 10000 ppm glyphosate acid were statistically significantly reduced from control values. In the absence of any dose response relationship across all groups this is considered not to be of toxicological significance.

Macroscopic findings: No macroscopic findings were observed attributable to the administration of glyphosate acid.

Microscopic findings: There was no microscopic pathology attributable to the administration of glyphosate acid.

Incidental findings included minor granulomatous/inflammatory lesions in lung, alimentary tract and lymph node associated with ascarid migration. Imperfect spermatogenesis and minimal secretory activity of the prostate were observed in several sexually immature males. Minimal cystitis manifest as infiltration of the mucosa by inflammatory cells and small

Weight at dosing: 7.8–8.9 kg (males); 7.2–7.9 kg (females)

Acclimation period:	13 days + 20 days pre-treatment period
Diet/Food:	125 C3 pelleted diet (SAFE, Villemoisson, Epinay-sur-Orge, France), approx. 300 g per day. Due to weight loss in three animals the amount for these dogs was increased to 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.
Water:	Tap water, <i>ad libitum</i>
Housing:	Individually in pens containing wood shavings for bedding, except when a urine sample was required. The dogs were group-housed once a week, by sex and dose group, after the last recording of clinical signs in the afternoon, until the next morning.
Environmental conditions:	Temperature: 20 ± 5 °C Humidity: 50 ± 20 % Air changes: approx. 12/hour 12 hours light/dark cycle

In life dates: 2005-09-27 to 2006-10-17

Animal assignment and treatment: In a chronic oral toxicity study groups of four beagle dogs per sex received daily doses of 0, 30, 125 and 500 mg/kg bw/day glyphosate technical in gelatine capsules for 52 consecutive weeks. The dose levels were selected based on results of a 13-week oral (capsule) toxicity study in dogs. Dose formulations were prepared weekly by adding the required amount to the capsules. The dosages were calculated based on minimum nominal active substance content of 950 g/kg glyphosate in the test item. Analyses of the test item showed a glyphosate content consistently above 95 %. Thus, no adjustment was considered necessary. Since the test item was added under GLP conditions, no additional analyses of dose formulations were deemed necessary.

Administrations of dose capsules were done approximately the same daily time each day. The low and mid-dose animals received one capsule per day, the high-dose and control dogs received three capsules per day. The quantity of dosage form applied to each animal was adjusted weekly based on the most recently recorded body weight.

Clinical observations: Observations for morbidity, and mortality were made twice daily. A check for clinical signs of toxicity was made at least once daily on all animals. In addition, a detailed clinical examination was performed once before start of treatment and weekly thereafter until termination.

Body weight: Individual body weights were recorded three times before group allocation, on Day 1 (prior to treatment) and at weekly thereafter during the conduct of study and at termination.

Food consumption and compound intake: Food consumption of each animal was estimated daily by noting the difference between the amount provided and the remaining amount on the next morning. Food consumption was expressed as percentage of quantity provided. Whenever fasting was required, food was removed at the end of the day and estimation of food consumption as made at that time.

Ophthalmological examination: Ophthalmological examinations were performed on all dogs prior to start and at the end of the treatment period. Pupillary light and blink reflexes were evaluated first. Mydriasis was then induced by adding Tropicamide solution into the eyes and the appendages, optic media and fundus were examined by indirect ophthalmoscopy.

Haematology and clinical chemistry: Blood samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. The following haematological parameters were examined: haemoglobin concentration (HB), erythrocyte count (RBC), mean cell volume (MCV), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), thrombocytes (PLAT), leukocytes (WBC), differential white cell count with cell morphology, neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L), monocytes M , reticulocytes (RETIC), prothrombin time (PT), and activated partial thromboplastin time (APTT). The following clinical chemistry parameters were examined: alkaline phosphatase (ALP), alanine aminotransferase activity (ALAT), aspartate amino transferase (ASAT), albumin, albumin/globulin ratio, total bilirubin, glucose, urea, calcium, chloride, total cholesterol, creatinine, γ -glutamyl-transferase (GGT), inorganic phosphorus, total protein, sodium, potassium, and triglycerides.

Urinalysis: Individual urine samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. Urine was collected in the presence of thymol crystals. The following examinations were made: appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen. The sediment was examined microscopically for leukocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium phosphate crystals, calcium oxalate crystals and cells.

Sacrifice and pathology: All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary, prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.

Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson's fixative, and testes and epididymides which were preserved in Bouin's fluid): adrenals, aorta, brain, caecum, colon, duodenum, oesophagus, eyes and optic nerve, epididymides, femur with articulation, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver, lungs with bronchi, mammary gland, mandibular lymph node, mesenteric lymph node, skeletal muscle, ovaries, oviducts, parathyroid, pancreas, pituitary, prostate, rectum, salivary glands (parotid and submandibular), skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum with bone marrow, stomach, sciatic nerve, testes, thymus, thyroids with parathyroid, tongue, trachea, urinary bladder, ureters, and uterus (horns and cervix).

A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics: Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002, ASB2013-3754), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

Results and discussion

Mortality: No mortalities or premature sacrifices occurred during the treatment period.

Clinical observations: There were no treatment-related clinical signs observed during the study period.

Observed clinical signs consisted of vomiting or soft faeces, thin appearance, hyperactivity, ptyalism, skin findings (scabs and erythema, generally localised on the ear(s)) and nodules on the ears. These clinical observations were seen transiently, and were encountered with a similar incidence in both control and treated animals and/or were independent to the administered dose-level and/or are commonly noted when a test item is given by gavage and/or were already present before the beginning of the treatment period.

Body weight: There was no treatment-related effect on body weight development. The lower mean body weight recorded in high dose males at the end of the treatment period was due to the lower mean body weight gain during the first month of the study (see Table B.6.3-32). Individual body weight changes were within the range of physiological variations. In addition, such body weight changes were observed in both control and treated dogs.

Table B.6.3-32: Mean body weight and body weight changes (kg)

	Males				Females			
Dose level (mg/kg bw/day)	0	30	125	500	0	30	125	500
Mean bw prior to start (day -1)	8.2	8.3	8.3	8.3	7.4	7.4	7.6	7.4
Weeks 1 – 4	+0.6	+0.3	+0.5	+0.2*	+0.3	+0.3	+0.3	+0.3
Weeks 4 – 26	+1.4	+0.9	+1.4	+1.1	+1.2	+1.1	+1.5	+1.6
Weeks 26 – 52	+0.9	+1.4	+1.1	+0.8	+0.6	+0.2	+0.5	+1.1
Weeks 1 – 52/53	+2.8	+2.6	+2.9	+2.0	+2.1	+1.6	+2.3	+3.0
Mean bw in week 52/53	11.2	11.0	11.2	10.5	9.6	9.2	10.0	10.6

*statistically significant from control ($p < 0.05$)

The weight loss of some dogs observed in the control, and low-dose group during some periods of the study were resolved when the daily food quantity was increased. Therefore, these changes were considered not test substance related.

Food consumption

There was no treatment-related effect on food consumption noted during the study.

The reduced food consumptions noted during the study was not considered test substance related, since they occurred only on some occasions and in control and treated dogs.

Due to weight loss one male each of the low and mid dose group, and one control female received 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.

Ophthalmology: There were no ophthalmological findings observed at the end of the study period.

Haematology: There were no treatment-related effects noted in the haematological parameters.

The significant differences observed for the activated partial thromboplastin time (\downarrow), MCHC (\downarrow) and eosinophil counts (\downarrow) in the treated animals when compared to control dogs were only slight and not dose-related.

Clinical chemistry: There were no treatment-related effects noted in the clinical chemistry parameters.

The significant differences observed for the inorganic phosphorous (↓), calcium (↓), protein (↓), glucose (↑), albumin/globulin ratio (↑) and AP values (↑) in treated animals when compared to control dogs were only slight and not dose-related.

Urine analysis: There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

Organ weights: There were no statistically significant differences in organ weights and organ to body weight ratios between control and treated dogs.

The statistically significant lower mean brain weight observed in males at 125 mg/kg bw/day was confined to this dose and, thus, no dose response was apparent. In addition, there were no macroscopic or histopathological findings noted in this organ. Thus, this finding is considered incidental.

Table B.6.3-33: Body/Brain weights and statistics

Dose group (mg/kg bw/day)	0	30	125	500
No of animals	4	4	4	4
Mean final body weight (g)	11165.0	10830.0	11090.0	10255.0
Mean brain weight (g)	87.41	80.06	73.96**	84.09
Mean % of bodyweight	0.78978	0.74484	0.67578	0.82550

** : DUNNETT'S TEST based on pooled variances at 1 % (**) level

Assigned control group(s): 1.

Gross pathology: There were no test substance related macroscopic findings observed in any animal of all dose groups.

Histopathology: There were no test substance related microscopic findings observed in any tissue sample of any dose group.

Conclusion by the Notifiers

Based on the study results the NOEL and NOAEL in beagle dogs after 1-year oral exposure to glyphosate technical is 500 mg/kg bw/day.

RMS comments:

This study is considered acceptable. It is agreed to set the NOAEL at the highest dose level of 500 mg/kg bw/day. It can be confirmed that the alterations in clinical chemistry parameters were mostly not statistically significant and, if so, did not show a dose response. The only possible exception is a lower blood calcium level in high dose males that was observed in other studies with glyphosate, too. However, without any concomitant findings, e.g. on bones, this perhaps treatment-related effects is not considered adverse.

This study was run in the same laboratory and under similar conditions as the 90-day study by [REDACTED] (2007, ASB2012-11454) in which severe adverse effects were seen upon treatment of Beagle dogs with glyphosate at a high dose level of 1000 mg/kg bw/day. It is clear now that these adverse reaction to treatment was in fact confined to an exaggerated dose level and that the NOAEL is higher than 300 mg/kg bw/day as established in that previous study.

2nd new one-year study in dogs ([REDACTED] 1997)

Reference: IIA, 5.3.4/02

Report: [REDACTED] 1997

HR-001: 12-Month Oral Chronic Toxicity Study in Dogs.

Data owner: Arysta LifeScience

Study No.: [REDACTED] 94-0157

Date: 1997-03-20

not published, ASB2012-11458

Guidelines:

Japan MAFF Guidelines 59 NohSan No.4200, 1985

U.S. EPA FIFRA Guidelines Subdivision F, 1984

OECD 409 (1981)

Deviations:

None

GLP:

yes

Acceptability:

See RMS comment

Materials and methods

Test material: Glyphosate technical

Identification: HR-001

Description: White crystals

Lot/Batch #: T-950308

Purity: 94.61 %

Stability of test compound: Not mentioned in the report

Vehicle and/
or positive control: None

Test animals:

Species: Dog

Strain: Beagle

Source: [REDACTED]

Age: 5 months

Sex: Males and females

Weight at dosing: 7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)

Acclimation period: 23 and 31 days for males and females, respectively

Diet/Food: Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day

Water: Tap water, *ad libitum*

Housing: Individually in stainless steel cages 83.5 x 90.0 x 80.0 cm

Environmental conditions: Temperature: 24 ± 2 °C

Humidity: 55 ± 10 %

Air changes: 15/hour

12 hours light/dark cycle

In life dates: 1996-03-05 to 1997-03-04

Animal assignment and treatment: Groups of 4 males and 4 females Beagle dogs received the test material by incorporating it into the basal diet at a level of 0, 1 600, 8 000 or 50 000 ppm for a period of 12 months.

Clinical observations: All animals were observed daily for clinical signs.

Body weight: Individual body weights were recorded at initiation of treatment, weekly from weeks 1 to 13, and every 4 weeks from weeks 16 to 52. In addition, final body weight was measured before necropsy.

Food consumption and compound intake: Food consumption of each animal was recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 52. Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:

$$\text{Food consumption} = [\text{Feeding amount (250g diet + 250g water)} - \text{food residue}] + 2$$

Chemical intake (mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.

Ophthalmological examination: Ophthalmological examinations were performed on all dogs prior to start of the treatment period. The following items were examined: eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, fundus.

Haematology and clinical chemistry: Blood samples were collected from all dogs prior to treatment, in weeks 25 and 52. The following haematological parameters were examined: Hematocrit, Hemoglobin concentration, Erythrocyte count, Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, platelet count, total leukocyte count.

All animals were subjected to blood biochemical examinations at weeks 26 and 52.

The following clinical chemistry parameters were examined alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ -glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), Potassium (K), chloride (Cl).

Urine analysis: Prior to initiation of treatment and at weeks 25 and 51, all animals were subjected to urinalysis on the following parameters: appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen.

Sacrifice and pathology: All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary, prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.

Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson's fixative, and testes and epididymides which were preserved in Bouin's fluid): brain, spinal cord, peripheral nerve, pituitary, thymus, thyroids with parathyroids, adrenals, tonsil, spleen, bone with marrow, lymph nodes, heart, aorta, tongue, buccal mucosa of oral cavity, pharynx, salivary glands, esophagus, stomach, liver with gallbladder, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder,

testes, prostate, penis, epididymides, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland, all gross lesions.

A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics: Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies" (OECD, 2002, ASB2013-3754), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

Results and discussion

Mortality: There were no deaths in any dose groups of either sex.

Clinical observations: In the 50 000 ppm group, loose stool was observed in 3 of 4 males and 4 of 4 females. The animals in the 8 000 and 1 600 ppm groups did not show the clinical sign at all. In the control group, only one animal in each sex showed it. Most of the animals in the 50 000 ppm group frequently showed the sign throughout the treatment period, whereas the occurrence in the suffering animals of the control group was restricted to a limited period.

For other clinical signs observed, the occurrence was sporadic in all dose groups, or the incidence was almost comparable among the dose groups.

Body weight: In the 50000 ppm groups of both sexes, retarded body weight gain became evident gradually as the study progressed. Consequently, the mean body weights in this group at termination of treatment were 6 % in males and 11 % in females lower than those in the controls. However, statistically significant differences in mean body weights were not observed throughout the treatment between the control and treated groups including the groups receiving 50000 ppm.

Food consumption: Decreased food consumption was noted for one female in the 1 600 ppm group at weeks 24, 28, and 52 and for another female in the same group at week 32. Consequently, group mean food consumption in this group was decreased at those weeks. However, food consumption in this group recorded at other weeks was comparable to that of the controls. Moreover, the averaged group mean food consumption through the treatment period was almost comparable between the 1 600 ppm and control groups of females.

All males in all dose groups and females except the above 2 animals in the 1 600 ppm group consumed whole amount of diet offered every day.

Group mean chemical intakes were calculated from group mean values of food consumption and body weight, and the nominal dose levels. The overall group mean chemical intakes (mg/kg/day) through the whole treatment period are presented in the table below:

Table B.6.3-34: Mean test substance intake

Dose level (ppm)	Test substance intake (mg/kg bw/day)	
	Male	Female
1 600	34.1	37.1
8 000	182	184
50 000	1203	1259

Ophthalmology: No remarkable ocular changes were detected in animals in any dose groups at week 52.

Haematology: Statistically significant changes in haematology that were observed in treated groups are presented in the following table:

Table B.6.3-35: Results of haematological examination

Dose level (ppm)		1 600			8 000			50 000		
Week of treatment		0	26	52	0	26	52	0	26	52
Sex	Female									
Parameter	Hematocrit (Ht)	-	-	-	-	-	-	↓	-	↓
	Hemoglobin concentration (Hb)	-	-	-	-	-	-	-	↓	↓
	Erythrocyte count (RBC)	-	-	-	-	-	-	↓	-	↓

↓: P<0.05 ; - : not significant, statistically evaluated by Dunnett's multiple comparison method.

Male groups showed no significant changes in any parameters.

Females in the 50 000 ppm group showed significantly decreased values of hematocrit (Ht), hemoglobin concentration (Hb), and erythrocyte count (RBC) at week 52. Hemoglobin concentration in this group was also significantly lower at week 26. This group had already showed lower values for these 3 parameters than the controls before initiation of treatment (at week 0). In particular, the differences from the control values in hematocrit and erythrocyte count at week 0 were statistically significant. However, the rates of deviation from the control values were, though slightly, augmented in the treatment period when compared to those at week 0.

Females in the 8 000 and 1 600 ppm groups showed no significant changes in hematological examinations

Clinical chemistry

Statistically significant changes in blood biochemistry that were observed in treated groups are presented in the table hereafter:

Table B.6.3-36: Results of clinical chemistry examination

Dose level (ppm)		1 600			8 000			50 000		
Week of treatment		0	26	52	0	26	52	0	26	52
Male										
Creatine phosphokinase (CPK)		-	-	-	-	-	↓	-	-	-
Female										
Albumin (Alb)		-	-	-	-	-	-	-	-	↓
Calcium (Ca)		-	-	↓	-	-	-	-	-	↓
Inorganic phosphorus (P)		-	-	-	-	-	-	-	-	↓
Chloride (Cl)		-	-	-	-	-	-	-	↑	-

↓: P<0.05 ; ↑: P<0.01 ; - : not significant, statistically evaluated by Dunnett's multiple comparison method.

Females in the 50000 ppm group showed a significant increase in chloride (Cl) at week 26 and significant decreases in albumin (Alb), calcium (Ca), and inorganic phosphorous (P) at week 52. A significant decrease in calcium was also noted for females in the 1 600 ppm group at 52 weeks.

For male groups, the 8000 ppm group showed a significant decrease in creatine phosphokinase (CPK) at week 52. But this change was not observed in the 50 000 ppm group.

Urine analysis: There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

Organ weights: Males in the 1 600 ppm group showed statistically significant increases in both absolute and relative weights of the pituitary. However, these changes were not observed in the 50 000 or 8 000 ppm groups of males.

In the 50 000 or 8 000 ppm groups, neither males nor females showed statistically significant changes in any organ weights

Gross pathology: The macroscopic lesions observed in the present study were all sporadic in nature and there were no statistically significant differences in the incidence between the control and treated groups.

Histopathology: In the 50 000 ppm group, focal pneumonia / focal granulomatous pneumonia in the lung was observed in all females. In the other female groups, including the control group, the lesion was observed in only one of 4 animals each. However, the extent of the lesions was slight in all cases including those of the 50 000 ppm group. Statistically, no significant differences between the control and dose groups were found in incidence of any histological lesions, including the pulmonary lesion.

Conclusion by the Notifiers

Based on the study results the NOEL in beagle dogs after 1-year oral exposure to HR-001 is 8000 ppm (equivalent to 182 and 184 mg/kg/day for males and females, respectively).

RMS comments:

The study is considered acceptable although there was an uncertainty about the dose levels that were actually tested. In the original study summary (p. 17), dose levels of 2000, 10000, and 30000 ppm are mentioned. According to a different information on the same page and in the following part of the report, dose levels were 1600, 8000, and 50000 ppm. It is assumed that the latter is correct but this error might provoke some doubts about the quality assurance system of the performing laboratory. The NOAEL (assumed to be 8000 ppm), however, is agreed with.

3d new one-year study in dogs ([REDACTED] 1996)

Reference: IIA 5.3.4/03

Report: [REDACTED] 1996 Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs

Data owner: Syngenta

Report No.: [REDACTED]/P/5079

Date: 1996-09-24

not published, TOX2000-1992

Guidelines: OECD 452 (1981): OPPTS 870.4100 (1998): 87/302/EEC B.30 (1988)

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Materials and methods

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	As given in report 95.6 % a.i
CAS#:	If available
Stability of test compound:	Confirmed by the Sponsor
Vehicle:	The test substance was administered in the diet.
Test Animals:	
Species	Dog
Strain	Beagle
Age/weight at dosing	20 – 29 weeks
Source	
Housing	Housed by treatment group (sexes separately) in indoor pens. The pens had a sleeping platform with heated floor underneath and interlinking gates which enable the dogs to be separated for feeding and dosing.
Acclimatisation period	4 – 5 weeks
Diet	Laboratory Diet A (Special Diet Services Ltd., Stepfield, Witham, Essex, UK) <i>ad libitum</i>
Water	Mains water <i>ad libitum</i>
Environmental conditions	Temperature: $19 \pm 2^{\circ}\text{C}$ Humidity: 40-70% Air changes: Approximately 15 changes / hour Photoperiod: 12 hours light / 12 hours dark

In-life dates: Start: 11 April 1995 End: 12 April 1996

Animal assignment: In a chronic toxicity study, groups of four male and four female beagle dogs were fed diets containing 0 (control), 3000, 15000, or 30000 ppm glyphosate acid, for a period of at least 1 year. A randomisation procedure was used which resulted in the even distribution of dogs (16 males and 16 females) to treatment groups according to body weight ensuring that litter mates were in different groups. Each morning, male dogs received 400 g and female dogs received 350 g of their appropriate experimental diet.

Table B.6.3-37: Study design

Test group	Dietary concentration (ppm)	Dose to animal (mg/kg) Males / females	# male	# female
Control	0		1 – 4	5 – 8
Low	3000	90.9 / 91.1	9 – 12	13 – 16
Mid	15000	440.3 / 447.8	17 – 20	21 – 24
High	30000	906.5 / 926.2	25 – 28	29 – 32

Diet preparation and analysis: The experimental diets were made in 60 kg batches, by direct addition of glyphosate acid (allowing for purity) to ground Laboratory A diet, and mixed thoroughly. Water was then added to each batch and mixed prior to pelleting. The pellets were dried in the residual heat of an autoclave, allowed to cool and were then stored in bins at room temperature.

Samples from all dietary levels (including controls) were taken at approximately two-monthly intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in Lab diet A was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet was determined over a period of up to 10 weeks (69 days) for these same diets.

Samples were extracted with water, portions of the supernatant were diluted with water to give sample solution concentrations within the range of the calibration standards. These were derivitised using 9-fluorenylmethylchloroformate (FMOCCL) and analysed by High Performance Liquid Chromatography (HPLC).

Observations: All dogs were observed at least three times daily for clinical behavioural abnormalities (at dosing, after dosing and at the end of the working day) and, on a weekly basis, they were given a thorough examination. Individual, daily assessments of gastro-intestinal findings were made for up to 5 hours post dosing: any subsequent assessments were made on a group basis. All dogs were also given a full clinical examination by a veterinarian pre-study, during weeks 13, 26, 39 and prior to termination. The examination included cardiac and pulmonary auscultation.

Body weight: All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals until termination.

Food consumption and test substance intake: Food residues were recorded daily, approximately 4 hours after feeding and any residual food was discarded. These measurements were made for at least 2 weeks pre-study and throughout the treatment period.

Ophthalmoscopic examination: The eyes of all dogs were examined pre-study, during weeks 13, 26, 39 and prior to termination.

Haematology: Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing EDTA or trisodium citrate and the following parameters measured.

haemoglobin	mean cell haemoglobin concentration
haematocrit	platelet count
red blood cell count	total white cell count
mean cell volume	differential white cell count
mean cell haemoglobin	red cell distribution width
prothrombin time	activated partial thromboplastin time
blood cell morphology	bone marrow smears (taken but not examined)

Clinical chemistry: Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing lithium heparin and the following parameters measured.

urea	alkaline phosphatase activity
creatinine	aspartate aminotransferase activity
glucose	alanine aminotransferase activity
albumin	gamma-glutamyl transferase activity
total protein	Calcium

cholesterol	phosphorus (as phosphate)
triglycerides	Sodium
total bilirubin	Potassium
creatine kinase activity	Chloride

Urinalysis: Urine was collected by catheterisation, pre-experimentally, in week 26 and during the week prior to termination. The following parameters were measured and recorded on each urine sample:

volume	Glucose
colour (if abnormal)	Ketones
specific gravity	Protein
pH	Bilirubin
	Blood

In addition, each urine sample was centrifuged and the sediment stained and examined microscopically to identify the components.

Macroscopic examination: All animals were killed by exsanguination under terminal anaesthesia induced by intravenous administration of sodium pentobarbitone and examined *post mortem*.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands	Kidney
Brain	Liver
Epididymides	Testes
thyroid glands	

The left and right components of paired organs were weighed separately.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

gross lesions including masses	oesophagus
adrenal gland	ovary
aorta	Pancreas
brain (cerebrum, cerebellum and brainstem)	parathyroid gland
bone marrow (sternum)	pituitary gland
caecum	prostate gland
colon	Rectum
duodenum	salivary gland
epididymis	spinal cord (cervical, thoracic, lumbar)
eyes (retina, optic nerve)	Skin
femur (including stifle joint, stored not examined)	Spleen
Gall bladder	Sternum
heart	Stomach

ileum	Testis
jejunum	Thymus
kidney	thyroid gland
Larynx	Trachea
liver	urinary bladder
lung	uterus (with cervix)
lymph node - prescapular	voluntary muscle
lymph node - mesenteric	
mammary gland (females only)	

Microscopic examination: All processed tissues were examined by light microscopy.

Statistics: All data were evaluated using analysis of variance and/or covariance for each specified parameter using the GLM procedure in SAS (1989).

Results and discussion

Concentration analysis results: The mean achieved concentrations of glyphosate acid in analysed dietary preparation were typically within 12 % of nominal concentration.

The overall mean concentrations were within 9 % of target.

Homogeneity results: The homogeneity of glyphosate acid in diet at concentrations of 3000 ppm and 30000 ppm for a batch size of 60 kg was determined and considered satisfactory; percentage deviations from the overall mean were within 11 %.

Stability results: The chemical stability of glyphosate acid in experimental diets (determined at concentrations of 3000 ppm and 30000 ppm) when stored at room temperature, was shown to be satisfactory for 69 days. This covered the period of usage on the present study.

Mortality: None of the dogs died.

Clinical observations: There were no toxicologically significant findings. Salivation at dosing was observed in individual dogs in all treatment groups throughout the study. The apparent increased incidence in two top dose males and one female, was considered to be related to anticipation of feeding and not to treatment with glyphosate acid. There was also a low incidence of scrotal skin reddening seen in one male in each treatment group; this was considered to be incidental to treatment with glyphosate acid.

There was no increased incidence of faecal abnormalities in dogs treated with glyphosate acid.

Bodyweight and weight gain: There was a slight bodyweight effect evident in females fed 30000 ppm glyphosate acid with a maximum reduction of 11 % (compared to controls) in week 51. These dogs showed a gradual reduction in growth rate, compared to the controls, which was consistently statistically significant from week 23 onwards. One female lost 0.6 kg during week 32 but this was related to a loss of appetite during this time. There were no effects in males at any dose level or in females at 15000 ppm but females fed 3000 ppm glyphosate acid also showed slightly poorer growth than the controls, with a maximum reduction of 8 % in week 51. However, this effect only achieved statistical significance on occasions during the study and is considered attributable to the poorer growth of two females and not an effect of glyphosate acid, since there was no effect at 15000 ppm.

Table B.6.3-38: Intergroup comparison of body weights (selected timepoints; adjusted mean values shown for weeks 2-14)

Dietary Concentration of Glyphosate acid (ppm)		
	Males	Females

week	0	3000	15000	30000	0	3000	15000	30000
1	11.40	11.53	11.33	11.45	9.60	9.55	9.48	9.58
8	12.66	12.40	12.48	12.37	10.74	10.40*	10.68	10.42*
16	13.35	12.97	13.28	12.95	11.46	11.03*	11.50	10.99*
32	14.19	13.69	13.93	13.69	12.28	11.63*	12.59	11.46**
53	14.57	14.24	14.24	13.85	13.10	12.25	12.94	12.76**
* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)								
** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)								

Food consumption: There was no effect on food consumption but 3 dogs left food on occasions which affected the group mean values:

Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg body weight. Mean values are shown below:

Table B.6.3-39: Mean Dose Received (mg/kg bw/day)

Glyphosate acid (ppm)	3000	15000	30000
Males	90.9	440.3	906.5
Females	91.1	447.8	926.2

Ophthalmoscopic examination: There was a very low incidence of corneal or lenticular opacities but these were seen in control animals as well as those fed glyphosate acid. There were no treatment related abnormalities.

Haematology: There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry: There were no toxicologically significant findings

Plasma cholesterol levels were increased slightly in the treated groups of both sexes at weeks 26 and 52 but there was no evidence of any dose relationship.

Plasma phosphorus levels were lower in the male treated groups at week 52 but this was due, in part, to slightly higher individual control values. Similarly the reduced sodium value in males fed 30000 ppm at week 52 was due solely to one male.

Various animals in all groups (including controls) showed evidence of higher plasma alanine aminotransferase, aspartate aminotransferase and creatine kinase activities throughout the study as well as pre-experimentally, but there was little evidence of any conclusive group effects.

Other statistically significant differences were minor and/or not dose related and were considered to be of no toxicological significance.

Urinalysis: There were no differences in urine clinical chemistry parameters which were considered to be related to treatment.

Organ weights: There were no treatment related effects on any organ weights. Adrenal weights were slightly raised in the male 3000 ppm group but this was exaggerated by a low value for one male in the control group.

Macroscopic findings: Several treated females showed red areas in or diffuse reddening of the urinary bladder mucosa. The incidence was not clearly related to dose and in the absence of a similar effect in males it was considered unlikely that the lesion is related to the administration of glyphosate acid.

Microscopic findings: It was considered unlikely that any of the lesions confined to the treated groups were related to the administration of glyphosate acid as they were either of low incidence or the incidence was not related to dose. The pathological no-effect level for glyphosate acid was 30000 ppm.

Conclusion by the Notifiers

Oral administration of 0, 3000, 15000 or 30000 ppm glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in bodyweight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg/day for males and 926 mg/kg/day for females.

There were no other treatment related findings and the pathological no-effect level was 30000 ppm glyphosate acid.

The no-observed effect level for toxicity over 1 year for females was 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg/day). The no-observed effect level for toxicity over 1 year for males was 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg/day).

RMS comments:

The study is considered acceptable. Based on the reductions in body weight gain in high dose females, the NOAEL was the mid dose level of 15000 ppm, i.e., 447 mg/kg bw/day.

Published information

Not available for dogs.

B.6.4 Genotoxicity (Annex IIA 5.4)

Introduction into this chapter by the RMS

In this section, only genotoxicity studies are reported in detail that were not contained in the original DAR (1998, ASB2010-10302) because they either had not been submitted for previous EU evaluation or were conducted more recently. The study descriptions, evaluations and tables as submitted for the new studies were amended where necessary and each of these studies was commented by the RMS. Redundant parts were deleted. The previously known studies from the 1998 DAR were re-evaluated and used only if considered still acceptable or at least supplementary. A detailed description of these studies and their results may be found in the old DAR. If studies were regarded now as “not acceptable”, they were only briefly mentioned and deleted from the summary tables.

A sub-section on mutagenicity of formulations was included that is mainly based on an Addendum to the original DAR that was prepared in 2000 (ASB2013-2748). In the last sub-section of this chapter, more recent publications dealing with mutagenicity of glyphosate or its formulations are discussed

Overall evaluation of genotoxicity is presented in Volume 1.

B.6.4.1 *In vitro* genotoxicity testing – Bacterial assays for gene mutation

In Table B.6.4-1, the available Ames tests of acceptable quality are summarised that had been submitted either for first EU evaluation in the 1990ies or, for the first time, for this re-evaluation. The “new” studies are reported below in detail and commented by the RMS. With regard to the “old” studies from the 1998 DAR (ASB2010-10302), most of them (in fact all but two) were deleted from current evaluation because, e.g., purity or batch number were not given, the concentrations were too low as compared to other studies or if they had been assessed as “supplementary” in the previous evaluation for other reasons.

Table B.6.4-1: Summary of valid *in vitro* genotoxicity tests with glyphosate acid in bacteria (Ames test)

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels; batch/lot number ^s , purity; metabolic activation	Results
Jensen, 1991; TOX9552371; Cheminova [#]	Ames test	<i>S. typhimurium</i> TA 98, 100, 1535, 1537	- S9: 160 – 2500 µg/plate; + S9: 310 – 5000 (plate-incorporation and pre-incubation test); Batch 206-JaK-25-1, 98.6%	Negative
Shirasu <i>et al.</i> , 1978; TOX9552368; Monsanto* [#]	Ames test	<i>S. typhimurium</i> TA 98, 100, 1535, 1537, 1538 and <i>E. coli</i> WP2 hcr	10 – 5000 µg/plate (plate-incorporation assay); Lot XHJ-46, 98.4%; +/- S9	Negative (supplementary study)
Akanuma, 1995; ASB2012-11462; Arysta	Ames test	<i>S. typhimurium</i> TA 98, 100, 1535, 1537 and <i>E. coli</i> WP <i>uvrA</i>	156-5000 µg/plate (pre-incubation test); 95.68%; +/- S9	Negative (supplementary study)
Sokolowski, 2007; ASB2012-11463; Nufarm	Ames test	<i>S. typhimurium</i> TA 98, 100, 1535, 1537 and <i>E. coli</i> WP <i>uvrA</i>	3 – 5000 µg/plate (plate-incorporation), 33 – 5000 µg/plate (pre-incubation test); 95.1%; +/- S9	Negative

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels; batch/lot number ^s , purity; metabolic activation	Results
Sokolowski, 2007; ASB2012-11464; Nufarm	Ames test	<i>S. typhimurium</i> TA 98, 100, 1535, 1537 and <i>E. coli</i> WP <i>uvrA</i>	3 – 5000 µg/plate (plate-incorporation) 33 – 5000 µg/plate (pre-incubation test); 97.7%; +/- S9	Negative
Sokolowski, 2007; ASB2012-11465; Nufarm	Ames test	<i>S. typhimurium</i> TA 98, 100, 1535, 1537 and <i>E. coli</i> WP <i>uvrA</i>	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	<i>S. typhimurium</i> TA 98, 100, 102, 1535, 1537	648 – 5000 µg/plate (plate-incorporation); 98.01%; +/- S9	Negative (supplementary study)
Flügge, 2009; ASB2012-11468; Helm	Ames test	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> TA 98, 100, 1535, 1537 and <i>E. coli</i> WP <i>uvrA</i>	3 – 5000 µg/plate (plate incorporation and pre-incubation test); 97.16% technical a.i. containing 0.63% glyphosine; +/- S9	Negative
Wallner, 2010; ASB2012-11471; Helm	Ames test	<i>S. typhimurium</i> TA 98, 100, 102, 1535, 1537	31.6 – 5000 µg/plate (plate incorporation and pre-incubation test); 98.2%; +/- S9	Negative
Thompson, 1996; ASB2012-11472; Nufarm	Ames test	<i>S. typhimurium</i> TA 98, 100, 1535, 1537 and <i>E. coli</i> WP <i>uvrA</i>	0 – 5000 µg/plate (plate-incorporation); 95.3%; +/- S9	Negative (supplementary study)
Callander, 1996; ASB2012-11473; Syngenta	Ames test	<i>S. typhimurium</i> TA 98, 100, 1535, 1537 and <i>E. coli</i> WP2P <i>uvrA</i> 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	<i>S. typhimurium</i> TA 98, 100, 1535, 1537 and <i>E. coli</i> WP2 <i>uvrA</i> pKM 101 and WP2 pKM 101	3 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 96.3%; +/- S9	Negative

^s Batch/lot numbers are only given for the “old” studies. For the “new” studies, they are mentioned in the comprehensive study descriptions below.

* Results were also published by Li & Long, 1988 (TOX9500253).

The study was reported in the 1998 DAR (ASB2010-10302) and, accordingly, was subject to previous EU evaluation yet.

Some of the studies mentioned in Table B.6.4-1 are considered supplementary because either the plate-incorporation method or the pre-incubation method was performed but not both. This assessment is in line with current guideline requirements but does not affect validity of the results obtained with the method used. For overall assessment of this end point, the available database is sufficient.

The studies by Thompson (1995, TOX9500251), Fassio (1995, TOX9551631), Suresh (1993, TOX9551098), Jenkinson (1990, TOX9500268) and Bhide (1986, TOX9551955) as well as the study with the IPA salt by Wang *et al.* (1993, TOX9500381) as reported in the 1998 DAR (ASB2010-10302) but were not considered acceptable by the RMS upon re-evaluation. Likewise, the more recent study by Miyaji (2008; ASB2012-11467) was considered not acceptable because the highest concentration level was much too low. However, it should be noted that also these less reliable tests of lower quality did not reveal any indications of genotoxicity.

1st new Ames test (Akanuma, 1995)

Reference: IIA, 5.4.1/01
Report: Akanuma, M., 1995a HR-001: Reverse mutation test.
 The Institute of Environmental Toxicology, Tokyo, Japan
 Data owner: Arysta LifeScience
 Study No.: IET 94-0142
 Date: 1995-04-03
 not published, ASB2012-11462
Guidelines: U.S. EPA FIFRA Guidelines, Subdivision F
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1995-02-21 to 1995-03-09

Materials and methods

Test material: Glyphosate technical
 Identification: HR-001
 Description: Solid crystals
 Lot/Batch #: 940908-1
 Purity: 95.68 %
 Stability of test compound: Not mentioned in the report
 Solvent used: Sterile water
 control materials:
 Negative: Sterile water
 Solvent/final concentration: Water / > 12 mg/mL
 Positive: non-activation
 and activation

Strain	Positive controls	
	Without S9 (µg/plate)	With S9 (µg/plate)
TA100	AF-2 (0.01)	2-AA (1)
TA1535	NaN ₃ (0.5)	2-AA (2)
WP2 uvrA	AF-2 (0.01)	2-AA (10)
TA98	AF-2 (0.1)	2-AA (0.5)
TA1537	9-AA (80)	2-AA (2)

AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide dissolved in DMSO; NaN₃ : sodium azide dissolved in sterile water

2-AA: 2-aminoanthracene dissolved in DMSO; 9-AA: 9-aminoacridine hydrochloride dissolved in sterile water

activation:

The enzyme activity measured by mutagenicity was good. S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl₂, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.

test organisms:

Escherichia coli WP2 uvrA
Salmonella typhimurium (TA100, TA1535, TA98 and TA1537)

Preliminary cytotoxicity assay:

Plate incorporation assay and pre-incubation assay: Concentrations up to 5000 µg/plate were evaluated with and without S9 activation in strain TA1535, TA1537, TA98, TA100 and WP2 uvrA. A single plate was used, per dose, per condition.

Mutation assays:

Plate incorporation assay: 156, 313, 625, 1250, 2500 and 5000 µg/plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.

Pre-incubation assay: As above for the plate incorporation assay.

The independently repeated mutation assay was conducted using the pre-incubation modification to the standard plate incorporation test only. The pre-incubation assay was carried out as described above with the following two exceptions: 0.5 mL of buffer were added to cultures prepared for testing under non-activated conditions; prior to the addition of top agar, reaction mixtures were incubated for 20 minutes at 37 ± 1 °C.

Statistics

Results were judged without statistical analysis.

Reproducibility of results was confirmed by two independent experiments.

Results were judged positive without statistical analysis when the following criteria are all satisfied:

A two-fold or greater increase above solvent control in the mean number of revertants is observed

This increase in the number of revertants is accompanied by a dose-response relationship

This increase in the number of revertants is reproducible.

Results and discussion

Analytical determinations: Not performed.

The results of the preliminary cytotoxicity assay are given in Table B.6.4-2.

Table B.6.4-2: Preliminary dose range finding test

		Dose	Revertants (n° colonies/plate)			
			Base-change type			Frameshift type
		(µg/plate)	TA100	TA1535	WP2uvr A	TA98 TA1537
- S9 mix	Solvent control (H ₂ O)		124 111 (118)	14 18 (16)	25 21 (23)	17 25 (21) 7 5 (6)
		200	113	16	24	20 7
		500	116	15	23	10 3
		1000	116	16	20	14 4
		2000	82	10	15	5 5
		5000	79	3	19	8 3
+ S9 mix	Solvent control (H ₂ O)		83 86 (85)	11 9 (10)	21 25 (23)	29 28 (29) 6 10 (8)
		200	99	11	20	28 9
		500	82	7	12	30 6
		1000	97	8	28	25 6
		2000	96	9	18	38 7
		5000	33	4	27	20 5
Positive controls	- S9 mix	Compound	AF-2	NaN ₃	AF-2	AF-2 9-AA
		µg/plate	0.01	0.5	0.01	0.1 80
		Revertants/plate	648 724 (686)	583 559 (571)	312 344 (328)	669 708 (689) 798 775 (787)
	+ S9 mix	Compound	2-AA	2-AA	2-AA	2-AA 2-AA
		µg/plate	1	2	10	0.5 2
		Revertants/plate	640 658 (649)	371 372 (372)	610 645 (628)	285 304 (295) 71 81 (76)

(): average

HR-001 did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.

Mutation assays

Results are shown in tables hereafter.

Table B.6.4-3: Summary data – experiment 1

		Dose	Revertants (n° colonies/plate)*			
			Base-change type			Frameshift type
		(µg/plate)	TA10	TA153	WP2uvr A	TA9 TA153
- S9 mix	Solvent control (H ₂ O)		117 0	12 5	21 A	37 8 3 7
		156	119	11	12	40 3
		313	117	11	16	42 4
		625	139	9	15	39 2
		1250	125	9	22	43 5
		2500	106	3	15	38 3
		5000	105	4	20	39 2
+ S9 mix	Solvent control (H ₂ O)		78	9	21	35 7
		156	83	6	19	36 9
		313	77	7	19	31 5

		Dose	Revertants (n° colonies/plate)*			
			Base-change type			Frameshift type
		(µg/plate)	TA10	TA153	WP2uvr	TA9 TA153
			0	5	A	8 7
		625	99	6	19	30 8
		1250	93	6	22	37 6
		2500	73	7	16	39 7
		5000	56	3	16	25 4
Positive controls	- S9 mix	Compound	AF-2	NaN ₃	AF-2	AF-2 9-AA
		µg/plate	0.01	0.5	0.01	0.1 80
		Revertants/plate	510	524	305	621 786
	+ S9 mix	Compound	2-AA	2-AA	2-AA	2-AA 2-AA
		µg/plate	1	2	10	0.5 2
		Revertants/plate	606	392	522	360 75

* values are the mean of three plate

Table B.6.4-4: Summary data – experiment 2

		Dose	Revertants (n° colonies/plate)*			
			Base-change type			Frameshift type
		(µg/plate)	TA10	TA153	WP2uvr	TA9 TA153
			0	5	A	8 7
- S9 mix		Solvent control (H ₂ O)	146	9	16	24 5
		156	137	10	18	18 7
		313	140	7	19	20 4
		625	136	8	17	18 3
		1250	136	7	15	15 3
		2500	144	6	18	10 3
		5000	117	10	14	9 4
			123	8	17	37 7
+ S9 mix		Solvent control (H ₂ O)	123	8	17	37 7
		156	112	7	15	32 10
		313	125	7	13	29 9
		625	123	8	20	35 9
		1250	107	7	14	28 9
		2500	89	7	19	20 8
		5000	67	4	17	17 4
			123	8	17	37 7
Positive controls	- S9 mix	Compound	AF-2	NaN ₃	AF-2	AF-2 9-AA
		µg/plate	0.01	0.5	0.01	0.1 80
		Revertants/plate	595	527	252	742 909
	+ S9 mix	Compound	2-AA	2-AA	2-AA	2-AA 2-AA
		µg/plate	1	2	10	0.5 2
		Revertants/plate	768	322	605	327 87

* values are the mean of three plates

Conclusion by the Notifiers

A two-fold or greater increase in the mean number of revertant colonies was not observed in any strain at any dose of HR-001 in the reverse mutation tests with or without metabolic activation. It is concluded that HR-001 is non mutagenic for bacteria under the conditions used with this experiment.

RMS comments:

No evidence of genotoxicity was obtained.

When the study description in the dossier was compared to the original study report, it was noted that the study director was Mie Akanuma. Erroneously, the first name had been mentioned in the dossier instead of the authors surname. More important, it must be clarified that, according to the study report, only the pre-incubation method was used where the plate-incorporation assay is not described and was apparently not performed. At least, the results given in Table B.6.4-3 and Table B.6.4-4 were obviously obtained by means of the pre-incubation method. Since only one of the methods was used, the study is considered supplementary.

2nd new Ames test (Sokolowski, 2007)

Reference:	IIA, 5.4.1/02
Report:	Sokolowski, A. 2007 <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> Reverse mutation assay with glyphosate technical (NUP-05068) RCC Ltd., Itingen, Switzerland Data owner: Nufarm RCC Study No.: 1061401 Date: 2007-03-16 Unpublished, ASB2012-11463
Guidelines:	The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC). Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. > Notification 12NohSan No. 8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.
Deviations:	None
GLP:	yes
Acceptability:	See RMS comment

Dates of experimental work: January 15, 2007 to January 25, 2007

Materials and methods

Test material:	
Identification:	Glyphosate technical (NUP-05068)
Description:	Crystalline powder, White
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Not specified
Vehicle/Controls	Vehicle = water
Negative/solvent control:	Concurrent untreated and solvent controls were performed. without metabolic activation: Sodium azide, NaN ₃
Positive control:	with metabolic activation: 2-aminoanthracene, 2-AA

Activation: Phenobarbital/J3-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats

Test organisms:: Histidine auxotrophic strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100) to prototrophy.

Culture medium: 8 9 Merck Nutrient Broth (MERCK, 0-64293 Darmstadt)
5 9 NaCl (MERCK, 0-64293 Darmstadt)

Test concentrations:: 0, 33, 100, 333, 667, 1000, or 5000 µg/plate with or without metabolic activation.g

Study conduct:

For each strain and dose level including the controls, three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),

500 µL 89 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),

100 µL Bacteria suspension (cf. test system, pre-culture of the strains),

2000 µL overlay agar

In the pre-incubation assay 100 µL test solution, 500 µL S9 mix and S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37°C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark.

Results and discussion

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation, with the exception of strain TA 1537, where a minor recuction in the number of revertants was observed at 5000 Iµg/plate without metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was slightly exceeded in the solvent control of strain WP2 uvrA with metabolic activation in experiment I. This minor deviation is judged to be based on biologically irrelevant fluctuations and has no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Conclusion by the Notifiers

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this end point.

RMS comments:

The study is considered acceptable. No evidence of a mutagenic response was obtained. The lower number of revertants in one experiment with TA 1537 might point to a weak cytotoxic effect of the test substance to this strain at a high concentration. In the past, similar observations were occasionally made with glyphosate from different sources at high concentrations (see DAR, 1998, ASB2010-10302).

3d new Ames test (Sokolowski, 2007)

Reference: IIA, 5.4.1/03
Report: Sokolowski, A. 2007 *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with glyphosate technical (NUP-05070) RCC Ltd., Itingen, Switzerland
 Data owner: Nufarm
 RCC Study No.: 1061402
 Date: 2007-03-16
 Unpublished, ASB2012-11464
Guidelines: The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).
 Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12NohSan No. 8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: January 15, 2007 to January 25, 2007

Materials and methods

Test material:
 Identification: Glyphosate technical (NUP-05070)
 Description: Crystalline powder White
 Lot/Batch #: 20060901
 Purity: 97.7 %
 Stability of test compound: Not specified
 Vehicle/Controls: Vehicle = water
 Negative solvent control: Concurrent untreated and solvent controls were performed.
 Positive control: without metabolic activation: Sodium azide, NaN₃
 with metabolic activation: 2-aminoanthracene, 2-AA
 Phenobarbital/J3-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
 Activation:

Test organisms:: Histidine auxotrophic strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100) to prototrophy.

Culture medium: 8 9 Merck Nutrient Broth (MERCK, 0-64293 Darmstadt)
5 9 NaCl (MERCK, 0-64293 Darmstadt)

Test concentrations:: 0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or without metabolic activation.g

Study conduct:
See Sokolowski, 2007, (ASB2012-11463) above.

Results and discussion

The plates incubated with the test item showed reduced background growth at 333 - 5000 and 2500 - 5000 in strains TA 1537 and TA 100, respectively. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. Minor toxic effects occurred at 5000 µg/plate in strain WP2 uvrA in the absence of metabolic activation in experiment I and in strain TA 98 with metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with glyphosate technical (NUP-05070) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was not quite reached in the untreated control of strain TA 1535 with and without metabolic activation in experiment II. These minor deviations (10 versus 11 colonies and 9 versus 10 colonies, respectively) are judged to be based on biologically irrelevant fluctuations in the number of colonies and have no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Conclusion by the Notifier

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this end point.

RMS comments:

The study is considered acceptable. No evidence of a mutagenic response was obtained. Cytotoxic effects, if occurring, were confined to high concentrations and certain bacterial strains.

4th new Ames test (Sokolowski, 2007)

Reference: IIA, 5.4.1/04

Report: Sokolowski, A. 2007 *Salmonella typhimurium* and *Escherichia coli*
Reverse mutation assay with Glyphosate technical (NUP-05067)
RCC Ltd., Itingen, Switzerland
Data owner: Nufarm
RCC Study No.: 1061403
Date: 2007-03-16

Guidelines: unpublished, ASB2012-11465
The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).
Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12NohSan No. 8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.

Deviations: None

GLP: yes

Acceptability: See RMS comment

Dates of experimental work: January 16, 2007 to January 25, 2007

Materials and methods

Test material:
Identification: Glyphosate technical (NUP-05067)
Description: Crystalline powder, white
Lot/Batch #: 0609-1
Purity: 95 %
Stability of test compound: Not specified
Vehicle/Controls: Vehicle = water
Negative/solvent control: Concurrent untreated and solvent controls were performed.
Positive control: without metabolic activation: Sodium azide, NaN₃
with metabolic activation: 2-aminoanthracene, 2-AA
Phenobarbital/J3-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
Activation: Histidine auxotrophic strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100) to prototrophy.
Test organisms:: 8 9 Merck Nutrient Broth (MERCK, 0-64293 Darmstadt)
Culture medium: 5 9 NaCl (MERCK, 0-64293 Darmstadt)
Test concentrations:: 0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or without metabolic activation.g

Study conduct:
See Sokolowski, 2007a (ASB2012-11463), above.

Results and discussion

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation, with the exception of strain TA 1537, where a minor reduction in the number of revertants was observed at 5000 µg/plate without metabolic

activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP 05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was slightly exceeded in the solvent control of strain WP2 uvrA with metabolic activation in experiment I. This minor deviation is judged to be based on biologically irrelevant fluctuations and has no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Conclusion by the Notifiers

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

RMS comments:

The study is considered acceptable. No evidence of a mutagenic response was obtained. Previously known evidence of cytotoxicity was confirmed but findings were confined to high concentrations and certain bacterial strains.

5th new Ames test (Ribeiro do Val, 2007,)

Reference: IIA, 5.4.1/05
Report: Ribeiro do Val, R. 2007 Bacterial reverse mutation test (Ames Test) for Glifosato Técnico Helm
 TECAM Tecnologia Ambiental Ltda., Brazil
 Data owner: HAG
 Report No.: 3393/2007-2.0AM-B
 Date: 2007-12-13
 Unpublished, ASB2012-11466
Guidelines: OECD 471
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 30/11/2007 – 03/12/2007

Materials and methods

Test material:
 Identification: Glifosato Técnico Helm
 Description: Solid
 Lot/Batch #: 2007091801
 Purity: 980.1 g/kg
 Stability of test compound: Stable (CIPAC MT 46, 54 °C, 14 days)

Vehicle and/
or positive control:

Negative control: vehicle (DMSO, 100 µL/plate)

Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	ICR 191 – Acridine
S9-	TA102	Mitomycin C
S9+	TA98; TA100; TA102; TA1535; TA1537	2-aminoanthracene

S9 = metabolic activation

Test organisms/cells:

Species:

S. typhimurium

Strain:

TA98; TA100; TA102; TA1535; TA1537

Source:

Moltex Inc. (Annapolis, MD, USA)

Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

Test concentrations:

Preliminary cytotoxicity test 8, 40, 200, 1000, 5000 µg/plate

Mutation assay: 648, 1080, 1800, 3000, 5000 µg/plate

Preliminary cytotoxicity assay: A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 8.0 and 5000.0 µg/plate.

Mutation study: The mutation study was performed with and without metabolic activation. Each sample was prepared by mixing 0.1 mL of test substance, 0.1 mL of a fresh bacterial suspension grown overnight, 0.5 mL S9 mix or substitution buffer (with / without metabolic activation) and 3.0 mL of top agar. Each suspension was incubated on selective agar plates for 72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 648 and 5000 µg/plate. The protein concentration of the S9 fraction was 34.9 mg/mL.

Liability check: The acceptance criteria of the assay were as follows:

The presence of background lawn in the test plates.

Spontaneous revertant colonies of the negative control were in the range reported in the literature (MARON. D.M, & AMES. B.N. Revised methods for the *Salmonella* mutagenicity test, Mutation Research. 113: 173-215, 1983) and established in the laboratory by historical control values.

Positive controls showed mutagenic activity in all tested strains.

Results and discussion

Preliminary cytotoxicity assay: None of the concentrations tested showed cytotoxic effects.

Mutation assays: No significant mutation rate was observed up to a concentration of 5000 µg of test item per plate.

Liability check: All acceptance criteria were met.

Conclusion by the Notifiers

The test material glyphosate technical was non-genotoxic.

RMS comments:

The study is considered supplementary because only the plate-incorporation assay was performed but not the pre-incubation method. Furthermore, E. coli strains were not included. No evidence of mutagenicity was obtained. According to the study report, some cytotoxicity occurred that became obvious by a lower number of revertants when the strains TA1537 (with metabolic activation) and TA102 (without) were treated at the highest concentration level of 5000 µg/plate.

6th new Ames test (Miyaji, 2008)

Reference: IIA, 5.4.1/06
Report: Miyaji, C. K. 2008 Evaluation of the mutagenic potential of the test substance glyphosate technical by reverse mutation assay in *Salmonella typhimurium* (Ames Test) Bioagri Laboratorios Ltda., Brazil
Data owner: HAG
Report No.: RF-3996.401.391.07
Date: 2008-09-15
Unpublished, ASB2012-11467
Guidelines: OECD 471
Deviations: None
GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 05/06/2008 – 30/06/2008

Materials and methods

Test material:
Identification: GLYPHOSATE TECHNICAL
Description: Solid
Lot/Batch #: 20070606
Purity: 980.5 g/kg
Stability of test compound: Stable to hydrolysis at pH 3, 6 and 9 (5-35 °C)
Vehicle and/or positive control: Vehicle: DMSO

Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA97a	9-Aminoacridine
S9-	TA102	Cumene hydroperoxide
S9+	TA97a; TA98; TA100; TA102; TA1535	2-Aminoanthracene

S9 = metabolic activation

Test organisms/cells:

Species:

S. typhimurium

Strain:

TA97a; TA98; TA100; TA102; TA1535

Source:

Moltox Toxicology, Inc.

Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

Test concentrations:

Preliminary cytotoxicity test 0.001, 0.01, 0.1, 1.0, 2.5 mg/plate

Mutation assay: 0.001, 0.01, 0.1, 0.5, 1.0 mg/plate

Preliminary cytotoxicity assay:

A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.001 and 2.5 µg/plate.

Mutation study:

The mutation study was performed with and without metabolic activation. Each sample was prepared by mixing the corresponding volume of test stock solution, of test substance, 0.1 mL of a fresh bacterial suspension grown overnight, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 0.001 and 1.0 µg/plate.

Liability check: The concentration of test item in the lowest and highest concentrated sample were determined by HPLC-UV

Results and discussion

Preliminary cytotoxicity assay: Only the highest concentration of 2.5 mg/plate showed cytotoxic effects. Therefore, the mutation assay was carried out with a maximum concentration of 1.0 mg/plate.

Mutation assays: No significant mutation rate was observed up to a concentration of 1.0 mg of test item per plate.

Liability check: The concentrations of the lowest and highest concentrations were confirmed.

Conclusion by the Notifier

The test material glyphosate technical was non-genotoxic.

RMS comments:

No evidence of mutagenicity was obtained in the plate-incorporation assay. However, since the highest concentration of 1000 µg/plate was much lower than in most other studies and since no apparent reason for not using higher dose levels was given, the study is considered not acceptable.

7th new Ames test (Flügge, 2009)

Reference: IIA, 5.4.1/07
Report: Flügge, C. 2009 Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (in vitro)
 LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany
 Data owner: HAG
 Report No.: LPT 23916
 Date: 2009-04-30
 Unpublished, ASB2012-11468
Guidelines: OECD 471
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 04/02/2009 – 27/02/2009

Materials and methods

Test material:
 Identification: Glyphosate TC
 Description: Solid, white powder
 Lot/Batch #: 20080801
 Purity: 988.0 g/kg
 Stability of test compound: Stable for two years at ambient temperature
 Vehicle and/
 or positive control: Negative control: Vehicle (*aqua ad iniectabilia*)
 Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	9-Aminoacridine
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA102; TA1537	2-Aminoanthracene
S9+	TA100; TA1535	Cyclophosphamide

S9 = metabolic activation

Test organisms/cells:

Species: *S. typhimurium*

Strain:	TA98; TA100; TA102; TA1535, TA1537
Source:	Dr. Bruce N. Ames
Metabolic activation system:	S9 (microsomal fraction of rat liver induced with Aroclor 1254)
Test concentrations:	
Preliminary cytotoxicity test	0.316, 1.0, 3.16, 10.0, 31.6, 100.0, 316.0, 1000.0, 3160.0, 5000.0 µg/plate
Mutation assay:	31.6, 100.0, 316.0, 1000.0, 3160.0 µg/plate

Preliminary cytotoxicity assay:

A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and / or reduction of revertants by more than 50 %.

Mutation study: Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 48-72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The protein concentration of the S9 fraction was 31.55 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37 °C for 20 min prior to mixing with agar and plating as described above.

Liability check: As quality criteria the genotypes, i.e. histidine and biotin requirement (his⁻ (bio⁻), deep rough character (rfa⁻), UV-sensitivity (uvr B⁻), Ampicillin resistance (pKM 101) and Ampicillin / Tetracycline resistance (pAQ1) (only strain TA102) of the test strains were regularly confirmed.

Results and discussion

Preliminary cytotoxicity assay: Cytotoxicity was noted at concentrations of 3160.0 and 5000.0 µg/plate. Therefore, the mutation assay was carried out with a maximum concentration of 3160.0 µg/plate.

Mutation assays: No mutagenic effect was observed for glyphosate TC up to the cytotoxic concentration of 3160.0 µg/plate in the two independent experiments with and without metabolic activation.

Liability check: The genotypes of the 5 strains used were confirmed regularly.

Conclusion by the Notifier

The test material glyphosate technical was non-genotoxic.

RMS comments:

The study is considered acceptable. The highest concentration in the mutagenicity assays was chosen because there was evidence of cytotoxicity at this and above dose levels demonstrated at least for the strain TA100. This approach is reasonable and dose selection is supported.

8th new Ames test (Flügge, 2010)

Reference: IIA, 5.4.1/08

Report: Flügge, C. 2010 Mutagenicity study of glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*)
LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG,
Hamburg, Germany
Data owner: HAG
Report No.: LPT 24880
Date: 2010-01-25
Unpublished, ASB2012-11469

Guidelines: OECD 471

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 15/10/2009 – 23/11/2009

Materials and methods

Test material:

Identification: Glyphosate TC

Description: Solid, white powder

Lot/Batch #: 20080801

Purity: 964.0 g/kg

Stability of test compound: Stable for two years at ambient temperature

Vehicle and/
or positive control:

Negative control: Vehicle (*aqua ad iniectabilia*)

Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	9-Aminoacridine
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA102; TA1537	2-Aminoanthracene
S9+	TA100; TA1535	Cyclophosphamide

S9 = metabolic activation

Test organisms/cells:

Species:	<i>S. typhimurium</i>
Strain:	TA98; TA100; TA102; TA1535, TA1537
Source:	Dr. Bruce N. Ames
Metabolic activation system:	S9 (microsomal fraction of rat liver induced with Aroclor 1254)
Test concentrations:	
Preliminary cytotoxicity test	0.316, 1.0, 3.16, 10.0, 31.6, 100.0, 316.0, 1000.0, 3160.0, 5000.0 µg/plate
Mutation assay:	31.6, 100.0, 316.0, 1000.0, 3160.0 µg/plate

Preliminary cytotoxicity assay:

A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and / or reduction of revertants by more than 50 %.

Mutation study:

Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 48-72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The protein concentration of the S9 fraction was 26.6 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37°C for 20 min prior to mixing with agar and plating as described above.

Liability check:

As quality criteria the genotypes, i.e. histidine and biotin requirement (his⁻) (bio⁻), deep rough character (rfa⁻), UV-sensitivity (uvr B⁻), Ampicillin resistance (pKM 101) and Ampicillin / Tetracycline resistance (pAQ1) (only strain TA102) of the test strains were regularly confirmed.

Results and discussion

Preliminary cytotoxicity assay: Cytotoxicity was noted at concentrations of 3160.0 and 5000.0 µg/plate. Therefore, the mutation assay was carried out with a maximum concentration of 3160.0 µg/plate.

Mutation assays: No mutagenic effect was observed for glyphosate TC up to the cytotoxic concentration of 3160.0 µg/plate in the two independent experiments with and without metabolic activation.

Liability check: The genotypes of the 5 strains used were confirmed regularly.

Conclusion by the Notifier

The test material glyphosate technical was non-genotoxic.

RMS comments:

The study is considered acceptable. The test substance proved non-genotoxic. The choice of the highest concentration is sufficiently explained. In addition, some precipitation was observed in the pre-test with TA100 at 5000 µg/plate.

9th new Ames test (Sokolowski, 2010)

Reference: IIA, 5.4.1/09

Report: Sokolowski, A. 2010 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine
Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany
Data owner: HAG
Report No.: 1332300
Date: 2010-04-07
Unpublished, ASB2012-11470

Guidelines: OECD 471

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 17/03/2010 – 22/03/2010

Materials and methods

Test material:

Identification: Glyphosate TC (5000 mg/L) spiked with glyphosine (32 mg/L)

Description: An aqueous solution of glyphosate technical grade active ingredient (purity 97.16 % w/w), containing 0.63 % (w/w) glyphosine in the technical grade active ingredient.

Lot/Batch #: 2009051501 (Glyphosate TC)

Purity: 971.6 g/kg

Stability of test compound: Stable for two years at ambient temperature

Vehicle and/or positive control: Negative control: Vehicle (deionised water)

Positive controls:

Assay	Strain	Compound
S9-	TA98, TA1537	4-nitro-o-phenylene-diamine
S9-	TA100; TA1535	Sodium azide
S9-	WP2 uvrA	Methyl methane sulfonate
S9+	TA98; TA100; TA1535; TA1537, WP2 uvrA	2-Aminoanthracene

S9 = metabolic activation

Test organisms/cells:

Species: *S. typhimurium*

Strain: TA98; TA100; TA1535, TA1537

Species: *Escherichia coli*

Strain: WP2 uvrA

Source: Trinova Biochem GmbH (35394 Gießen, Germany)

Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)

Test concentrations:

Preliminary cytotoxicity test 3; 10; 33; 100; 333; 1000; 2500; 5000 µg/plate

Mutation assay: 3; 10; 33; 100; 333; 1000; 2500; 5000 µg/plate

Preliminary cytotoxicity assay:

A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and/or reduction of revertants.

Mutation study:

Each sample was prepared by mixing 1.0 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or S9 substitution buffer (with / without metabolic activation) and 1.0 mL of top agar. Each suspension was incubated on selective agar plates for at least 48 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The protein concentration of the S9 fraction was 34.3 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37 °C for 60 min prior to mixing with agar and plating as described above.

Liability check:

The acceptance criteria of the assay were as follows:

Regular background growth in the negative and solvent control.

Spontaneous revertant colonies of the negative control were in the range of historical data.

Positive controls showed mutagenic activity in all tested strains.

Results and discussion

Preliminary cytotoxicity assay: No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

Mutation assays: No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Solution of glyphosate TC spiked with Glyphosine at any dose level, neither in the presence nor absence of metabolic activation (S9 mix).

Liability check: The acceptance criteria were met.

Conclusion by the Notifiers

The test material glyphosate technical was non-genotoxic.

RMS comments:

The study is considered acceptable. No evidence of mutagenicity was obtained. The reason for glyphosine spiking of the test material is not clear but it is assumed that this substance (similar to glyphosate) may occur as an impurity in the technical active ingredient. Thus, this test might become particularly important if a certain specification needs to be assessed from a toxicological point of view.

10th new Ames test (Wallner, 2010)

Reference: IIA, 5.4.1/10
Report: Wallner, B. 2010 Reverse Mutation Assay using bacteria (*Salmonella typhimurium*) with Glyphosate TC
 BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany
 Data owner: Helm AG
 Report No.: BSL 101268
 Date: 2010-04-08
 Unpublished, ASB2012-11471
Guidelines: OECD 471
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 25/03/2010 – 06/04/2010

Materials and methods

Test material:

Identification: Glyphosate TC

Description: Solid.

Lot/Batch #: 200903051
Purity: 982.0 g/kg
Stability of test compound: Stable for two years at ambient temperature
Vehicle and/or positive control: Negative control: Solvent controls, consisting of solvent or vehicle alone.

Positive controls:

Assay	Strain	Compound
S9-	TA98, TA1537	4-nitro-o-phenylene-diamine
S9-	TA100; TA1535	Sodium azide
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA100; TA102; TA1535; TA1537	2-Aminoanthracene

S9 = metabolic activation

Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA102; TA1535, TA1537
Source: MOLTOX, INC, NC 28607, USA

Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)

Test concentrations:

Preliminary cytotoxicity test 31.6, 100, 316, 1000, 2500 and 5000 µg/plate

Mutation assay: 31.6, 100, 316, 1000, 2500 and 5000 µg/plate

Preliminary cytotoxicity assay:

No preliminary cytotoxicity assay was performed.

Mutation study:

Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or S9 substitution buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for at least 48 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The protein concentration of the S9 fraction was 33.0 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37 °C for 60 min prior to mixing with agar and plating as described above.

Liability check:

The acceptance criteria of the assay were as follows:

The bacteria demonstrate their typical responses to ampicillin (TA 98, TA 100, TA 102).

Regular background growth in the negative and solvent control.

Spontaneous revertant colonies of the negative control were in the range of historical data.

Positive controls showed mutagenic activity in all tested strains.

Results and discussion

Preliminary cytotoxicity assay: No preliminary cytotoxicity assay was performed.

Mutation assays: In the plate incorporation test toxic effects of the test item were observed in tester strain TA 100 at a dose of 5000 µg/plate (with and without metabolic activation). In tester strain TA 1535 toxic effects of the test item were noted at doses of 2500 µg/plate and higher (with and without metabolic activation). In the preincubation test toxic effects of the test item were noted in tester strains TA 100 and TA 1535 at a dose of 5000 µg/plate (without metabolic activation).

No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with Glyphosate TC at any concentration level with or without metabolic activation.

Liability check: The acceptance criteria were met.

Conclusion by the Notifiers

The test material glyphosate technical was non-genotoxic.

RMS comments:

The study is considered acceptable. Glyphosate proved non-genotoxic. Cytotoxicity was confined to very high concentrations.

11th new Ames test (Thompson, 1996)

Reference:	IIA, 5.4.1/11
Report:	Thompson, P.W. 1996 Technical glyphosate: Reverse mutation assay "Ames test" using <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> SafePharm Laboratories, Derby, UK Data owner: Nufarm SPL Project No.: 434/014 Date: 1996-02-20 unpublished, ASB2012-11472
Guidelines:	OECD 471 (1983) Commission Directive (EC) 92/69/EEC (1992), Method B14 US EPA (TSCA) guidelines
Deviations:	None
GLP:	yes
Acceptability:	See RMS comment

Dates of experimental work: August 19, 1995 to November 13, 1995

Materials and methods

Test material:

Identification: Technical Glyphosate

Description: White powder

Lot/Batch #: H95D 161 A

Purity: 95.3 %

Stability of test compound: No data given in the report.

Vehicle/Controls Vehicle = sterile distilled water

Negative/solvent control: Vehicle/solvent controls were performed.
Without metabolic activation:
N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):
- 2 µg/plate for WP2uvrA;
- 3 µg/plate for TA100;
- 5 µg/plate for TA1535.
9-Aminoacridine (9AA) 80 µg/plate for TA1537.
4-Nitroquinoline-1-oxide (4NQO) 0.2 µg/plate for TA98.

Positive control:

With metabolic activation:
2-Aminoanthracene (2AA) was used in S9 series of plates
in the concentrations:
- 1 µg/plate for TA100;
- 2 µg/plate for TA1535 and TA1537;
- 10 µg/plate for WP2uvrA;
- 0.5 µg/plate for TA98.

Activation:

S9 was prepared from the livers of male Sprague-Dawley rats. Each received a single i.p. injection of Aroclor 1254 at 500 mg/kg, 5 days before S9 preparation.

Test organisms: *Salmonella typhimurium* TA1535, TA 1537, TA98, and TA100

Escherichia coli WP2uvrA

Culture medium: Top agar was prepared using 0.6 % Difco Bacto agar and 0.5 % sodium chloride with 5 mL of 1.0 mM histidine/1.0 mM biotin and 1.0 mM tryptophan solution added to each 100 mL of top agar.

Base agar plates were prepared using 1.2 % Oxoid Agar Technical No 3 with Vogel-Bonner Medium E and 20 mg/mL D-glucose.

Test concentrations:

0, 50, 150, 500, 1500, and 5000 µg/plate with and without metabolic activation. In triplicate for each bacterial strain and for each concentration of test material with and without S9-mix.

Study conduct:

For each strain and dose level including the controls, three plates were used with and without S9-mix.

The following materials were mixed in a test tube and poured onto the selective agar plates:

0.1 mL of bacterial suspension;

0.1 mL of test solution at each dose level, vehicle/solvent (negative control) or reference mutagen solution (positive control);

2 ml of molten, trace histidine/tryptophan supplemented media;

0.5 ml of buffer (for test without metabolic activation) or S9-mix (for test with metabolic activation).

Known aliquots (0.1 mL) of one of the bacterial suspensions were dispensed into sets of sterile test tubes followed by 2.0 ml of molten trace histidine/tryptophan supplemented top agar at 45 °C, 0.1 mL of the appropriately diluted test material or vehicle control or reference mutagen solution (with and without metabolic activation) and either 0.5 mL of the S9 liver microsome mix or 0.5 ml of pH 7.4 buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner agar plates (one tube per plate). Plates were incubated for approximately 48 hours at 37 °C.

Results and discussion

The plates incubated with the test item caused no visible reduction in the growth of the bacterial lawn at any dose level up to maximum recommended dose of 5000 µg/plate either with or without metabolic activation, however a decrease in the frequency of revertant colonies was observed with some bacterial strains.

No significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains with any dose of the test material, either with or without metabolic activation. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

Conclusion by the Notifiers

In conclusion, the test material was found to be non-mutagenic under the conditions of this test.

RMS comments:

There was no evidence of mutagenicity obtained although there was some cytotoxicity. The study is considered supplementary since only the plate incorporation method was applied for testing.

12th new Ames test (Callander, 1996)**Reference:**

IIA, 5.4.1/12

Report:

Callander, R.D. 1996 Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E. Coli*.

Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

Data owner: Syngenta

Report No.: CTL/P/4874

Date: 1996-02-16

not published; ASB2012-11473

Guidelines:

OECD 471 (1997): OPPTS 870.5100 (1998): 2000/32/EEC

B.13/B.14 (2000)

Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1995-10-23 to 1996-02-16

Materials and methods

Test Material: Glyphosate acid
Description: Technical; white solid
Lot/Batch number: P24
Purity: 95.6 % a.i
CAS#: Not reported
Stability of test compound: Confirmed by Sponsor

Control Materials:

Negative: Dimethylsulphoxide – DMSO
Solvent control Dimethylsulphoxide – DMSO (10 µL/plate)
(final concentration):

Positive control:

Nonactivation:

Acridine mutagen ICR191 TA1537
2-Aminoanthracene TA1537, WP2 uA
Daunomycin hydrochloride TA98
N-Eethyl-N'-nitro-N-nitrosoguanidine WP2P *uvrA*
Mitomycin C WP2P
Sodium Azide TA1535 and TA100

Activation:

2-Aminoanthracene TA1535, TA1537, TA98, TA100,
WP2 *uvrA* and WP2P

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

The metabolic activation system (S9-mix) used in this study was prepared as a 3:7:20 mixture of S9 fraction, Sucrose-tris-EDTA buffer (250:50:1 mM) and cofactor solution.

The cofactor solution was prepared as a single stock solution Na₂HPO₄ (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and MgCl₂ (12 mM), in sterile deionised water and adjusted to a final pH of 7.4.

Test organisms:

<i>S. typhimurium</i> strains									
	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		
<i>E. coli</i> strains									
X	WP2P (pKM101)	X	WP2P <i>uvrA</i> (pKM101)						
Properly maintained?							X	Yes	No
Checked for appropriate genetic markers (<i>rfa</i> mutation, R factor)?							X	Yes	No

Test compound concentrations used:

Nonactivated conditions: 5000, 2500, 1000, 500, 200 and 100 µg/plate

Activated conditions: 5000, 2500, 1000, 500, 200 and 100 µg/plate

For all strains triplicate plates were used for all test substance and positive control treatments.

For solvent controls 5 plates were used.

Test performance

Preliminary Cytotoxicity Assay: Not performed.

Type of Bacterial assay:

X standard plate test (both experiments –S9, initial experiment +S9)

___ pre-incubation (60 minutes) (second experiment +S9)

___ “Prival” modification (i.e. azo-reduction method)

___ spot test

___ other

Protocol:

Bacterial cultures were prepared from frozen stocks by incubating for 10-12 hours at 37 °C.

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µL Test solution at each dose level, solvent and positive controls;

500 µL S9 mix or phosphate buffer;

100 µL Bacteria suspension;

2 mL Overlay agar containing 50 µM histidine or tryptophan as appropriate.

In this assay 100 µL aliquots of an overnight culture of each bacteria strain were stored in bijou bottles at room temperature until required (1-2 hours). 500 µL S9 mix (or buffer) was then added by dispensing syringe to the number of bijou bottles of one strain required for one dose level, followed by 0.1 mL of the appropriate concentration of the test substance solution added by micropipette. Finally, 2.0 mL top agar was added to each bijou, the force of addition was sufficient to mix the contents. The mixture was then rapidly poured onto a prepared Vogel Banner agar plate. After the agar was set the plates were incubated upside down for 64 - 68 hours at 37 °C in the dark. For each strain and dose level including the controls, three plates were used.

Following the total incubation period the plates were examined for the lack of microbial contamination and evidence that the test was valid: i.e. there should be a background lawn on the negative control plates and on the plates for (at least) the lower doses of test substance, and that the positive control had responded as expected.

The plates were counted using an automated colony counter (AMS 40-10) with the discrimination adjusted appropriately to permit the optimal counting of mutant colonies.

Statistical analysis: None – see Evaluation Criteria below.

Evaluation criteria: A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met:

a significant, dose-related increase in the mean number of revertants is observed;

a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) is observed at one or more concentrations

A negative result in a (valid) individual experiment is achieved when:

there is no significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance; and

in the absence of any such dose response, no increase in colony numbers is observed (at any test concentration) which exceeds 2x the concurrent solvent control.

For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain/S9 combination, then the observed effect(s) must be consistently reproducible.

Results and discussion

Mutagenicity assay: In two separate experiments, glyphosate acid did not induce any significant increases in the observed numbers of revertant colonies in the four *Salmonella typhimurium* strains (TA1535, TA1538, TA98, TA100) and the two *Escherichia coli* strains (WP2P and WP2 *uvrA*) in either the presence or absence of an auxiliary metabolising system (S9).

The positive controls for each experiment induced the expected responses indicating the strains were working satisfactorily in each case.

Conclusion by the Notifiers

Under the conditions of this assay, glyphosate acid gave a negative, ie non-mutagenic, response in *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* strains WP2P and WP2 *uvrA* in both the presence and absence of S9-mix, when tested to a limit dose of 5000 µg/plate.

RMS comments:

The study is considered acceptable. No evidence of mutagenicity was revealed. It should be clarified that the first experiment was performed by means of the plate incorporation method with and without metabolic activation. In the second trial, the same method was used in the absence of S9 mix. A pre-incubation assay was run with S9 mix.

13th new Ames test (Sokolowski, 2009)

Reference: IIA, 5.4.1/13

Report: Sokolowski 2009 Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay
Harlan Cytotest Cell Research GmbH, In den Leppsteinswiesen 19,
64380 Rossdorf Germany
Data owner: Syngenta
Report No.: 1264500

Guidelines: Date: 2009-12-18
 not published; ASB2012-11474
 OECD 471 (1997): OPPTS 870.5100 (1998): 2008/440/EC
 B.13/B.14 (2008)
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 2009-09-15 to 2009-12-18

This study was performed to investigate the potential of Glyphosate technical (produced via the Nantong Jiangshan (glycine-route)) to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and the *Escherichia coli* strains WP2 *uvrA* pKM 101 and WP2 pKM 101 over the range 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate (Experiment I), and 33; 100; 333; 1000; 2500; and 5000 µg/plate (Experiment II).

Materials and methods

Test Material: Glyphosate
Description: white solid
Lot/Batch number: 569753
Purity: 96.3 % of glyphosate acid
Stability of test compound: Not indicated by the sponsor

Control Materials:
Negative: Concurrent untreated and solvent controls were performed
Solvent control 100 µL/plate
(final concentration):
Positive control: Nonactivation:
 Sodium azide 10 µg/plate TA100, TA1535
 4-nitro-o-phenylene-diamine,
 50 µg/plate TA 1537, 10 µg/plate TA98
 methyl methane sulfonate 3 µL/plate WP2 (pKM101),
 WP2 *uvrA* (pKM101)
 Activation:
 2-Aminoanthracene
 2.5 µg/plate TA 1535, TA 1537, TA100, TA98
 10 µg/plate WP2 (pKM101), WP2 *uvrA* (pKM101)

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 10 % v/v in the S9 mix. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix:

8 mM MgCl₂
 33 mM KCl
 5 mM Glucose-6-phosphate
 5 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath.

Test organisms:

<i>S. typhimurium</i> strains									
	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		
<i>E. coli</i> strains									
X	WP2 (pKM101)	X	WP2 uvrA (pKM101)						
Properly maintained?							X	Yes	No
Checked for appropriate genetic markers (rfa mutation, R factor)?							X	Yes	No

Test compound concentrations used

The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II 33; 100; 333; 1000; 2500; and 5000 µg/plate

Test performance

Preliminary Cytotoxicity Assay

Not performed.

Type of Bacterial assay

X standard plate test (pre-experiment/experiment I; –S9, +S9)

X pre-incubation (60 minutes) (second experiment ; –S9, +S9)

___ “Prival” modification (i.e. azo-reduction method)

___ spot test

___ other

Protocol:

For each strain and dose level including the controls, three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),

500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),

100 µL Bacteria suspension (cf. test system, pre-culture of the strains),

2000 µL Overlay agar

In the pre-incubation assay 100 µL test solution, 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspensions were mixed in a test tube and shaken at 37° C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark.

* Substitution buffer: 8.5 parts of the 100 mM sodium-ortho-phosphate-buffer pH 7.4 with 1.5 parts of KCl solution 0.15 M

Statistical analysis:

None - see Evaluation Criteria below.

Evaluation criteria:

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

Results and discussion

Preliminary Cytotoxicity Assay : Not performed.

Mutation assays: Glyphosate technical was assessed for its potential to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and the *Escherichia coli* strains WP2 uvrA pKM 101 and WP2 pKM 101.

The assay was performed with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment / Experiment I:	3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:	33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No precipitation of the test item occurred up to the highest investigated dose.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with glyphosate technical at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

Conclusion by the Notifiers

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, glyphosate technical did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

RMS comments:

The study is considered acceptable. It could be shown that also technical glyphosate was not mutagenic when manufactured via the Nantong Jiangshan (glycine) route.

B.6.4.2 *In vitro* genotoxicity testing – Tests for gene mutation in mammalian cells

In contrast to the numerous Ames tests in bacteria, only few gene mutation assays with glyphosate have been performed in mammalian cells. An overview on the available information is given in Table B.6.4-5

Table B.6.4-5: Summary of *in vitro* genotoxicity tests with glyphosate acid in mammalian cells

Reference, study identification, owner		Type of study	Test organism / test system	Dose levels, batch no., purity, metabolic activation	Results
<i>In vitro</i> tests for gene mutation in mammalian cells					
Studies from the 2001 evaluation	Jensen, 1991; TOX9552372; Cheminova	Mouse lymphoma test	Mouse lymphoma cells (L5178Y)	- S9: 0.61 – 5.0 mg/mL, + S9: 0.52 – 4.2 mg/mL; 206-JaK-25-1; 98.6%	Negative
	Li, 1983*; TOX9552369; Monsanto	HGPRT assay	Chinese hamster ovary (CHO) cells	- S9: 2 – 22.5 mg/mL + S9: 5 – 22.5 (25 ??) mg/mL; Lot XHJ-64; 98.7%	Negative
New study	Clay, 1996; TOX2000-1994; Syngenta	Mouse lymphoma test	Mouse lymphoma cells (L5178Y TK ^{+/+})	+/- S9: 296 – 1000 µg/mL; P24; 95.6%	Negative

* Results were also published by Li & Long, 1988 (TOX9500253).

The previously known studies by Jensen (1991, TOX9552372) is reported in detail in the old DAR (1998, ASB2010-10302) and was confirmed to be acceptable upon re-evaluation. In this assay there was neither evidence of gene mutation (TK locus) nor of chromosome aberrations. A similar study (Clay, 1996, TOX2000-1994) confirming the absence of gene mutation and chromosome aberration in mouse lymphoma cells *in vitro* is described in the following in detail because it had not been submitted for previous EU evaluation. A negative HGPRT test (Li, 1983, TOX9552369) mentioned in the old DAR is also considered still

acceptable although it is not entirely clear from the original study report which dose level was actually the highest under activation conditions.

Mouse lymphoma assay (Clay, 1996)

Reference: IIA, 5.4.3/01
Report: Clay, P. 1998 Glyphosate Acid: L5178Y TK^{+/−} Mouse Lymphoma Gene Mutation Assay
Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK
Data owner: Syngenta
Report No.: CTL/P/4991
Date: 1996-05-24
not published, TOX2000-1994
Guidelines: OECD 476 (1997): OPPTS 870.5300 (1998): 2000/32/EEC B.17 (2000)
Deviations: The stability, homogeneity and achieved concentration of the test or control substances in the vehicle used were not determined by analysis and the certified purity and stability of the control substances are not available.
These deviations from the current regulatory guideline are considered not to compromise the scientific validity of the study..
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1996-01-29 to 1996-05-24

Materials and methods

Test Material: Glyphosate acid
Description: Technical, white solid
Lot/Batch number: P24
Purity: 95.6 % w/w a.i
CAS#: Not reported
Stability of test compound: Confirmed by the Sponsor

Control Materials:
Negative: DMSO
Solvent control: 1 %
(final concentration):
Positive control: Absence of S9 mix: Ethylmethanesulphonate (EMS), 750 µg/mL
Presence of S9 mix N-nitrosodimethylamine (NDMA), 600 µg/mL

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbital		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

X indicates those that apply

The co-factor solution was prepared as a stock solution of 75 mM NADP (disodium salt) and 1200 mM glucose-6-phosphate (monosodium salt) in RPMI 1640 culture medium with a final pH adjusted to 7.5. S9 fraction was added at 5 % (1 mL S9 added to the 20 mL cell culture) and co-factors at 1 % (200 µL to the 20 mL cell culture).

Test cells: Mammalian cells in culture

X	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		List any others:
Media: RPMI 1640			
Properly maintained?		X	Yes
Periodically checked for Mycoplasma contamination?		X	Yes
Periodically checked for karyotype stability?			Yes
Periodically "cleansed" against high spontaneous background?			Yes

X indicates those that apply

Locus Examined:		Thymidine kinase (TK)		Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na ⁺ /K ⁺ ATPase
Selection agent:		Bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		Fluorodeoxyuridine (FdU)		6-thioguanine (6-TG)		
	X	Trifluorothymidine (TFT)				

X indicates those that apply

Test compound concentrations used:	
Absence of S9 mix	296, 444, 667, 1000 µg/mL
Presence of S9 mix	296, 444, 667, 1000 µg/mL

Cells were exposed to test compound, negative/solvent or positive controls for 4 hours in both the presence and absence of S9 mix.

After washing, cells were cultured for 2 days (expression period) before cell selection.

After expression, 10⁴ cells/mL were dispensed, at 200 µL/well, into 96 well plates. The cells were cultured for 10-13 days in selection medium to determine numbers of mutants. Dilutions of the cultures to approximately 8 cells/mL were cultured for 10-13 days without selective agent to determine cloning efficiency.

Cell growth in individual microwell plates was assessed after 10-13 days using a dissecting microscope. The survival plates and viability plates were scored for the number of wells containing no cell growth (negative wells). The mutation plates were scored so that each well contained either a small colony (considered to be associated with clastogenic effects), a large colony (considered to be associated with gene mutation effects), or no colony.

Statistical methods: None required.

Evaluation criteria: Each well of the mutation plates (those containing TFT) was scored as containing either, a small colony, a large colony or no colony according to the following criteria:

Small Colony - a small colony was one whose average diameter was less than 25 % of the diameter of the well and was usually around 15 % of the diameter of the well. A small colony should also have shown a dense clonal morphology.

Large Colony - a large colony was one whose average diameter was greater than 25 % of the diameter of the well. A large colony should also have shown less densely packed cells, especially around the edges of the colony.

Any well which contained more than one small colony was scored as a small colony. Any well which contained more than one large colony was scored as a large colony. Any well which contained a combination of large and small colonies was scored as a large colony.

An empty well was one which contained no cell growth.

Results and discussion

Preliminary toxicity assay: The maximum concentration of glyphosate acid considered appropriate for testing in the mutation experiments was estimated to be 1000 µg/mL as concentrations of 1500 µg/mL and 2000 µg/mL in the presence and absence of S9-mix were found to produce an excessive reduction in the pH of the treatment medium. Very little toxicity was seen at the concentrations tested.

Mutation assay: No significant increases in mutant frequency, compared to the solvent control cultures, were observed in cultures treated with glyphosate acid at any concentration tested in either the presence or absence of S9-mix.

The positive controls, EMS and NDMA, induced substantial increases in mutant frequency in all mutation experiments, demonstrating the activity of the S9-mix and that the assay was performing satisfactorily in being capable of detecting known mutagens.

Conclusion by the Notifiers

Glyphosate acid was not mutagenic to L5178Y TK^{+/+} cells in the presence or absence of S9-mix.

RMS comments:

The study is considered acceptable and the conclusion is agreed with. The selection of the highest concentration of 1000 µg/mL because of pH reduction at dose levels above is reasonable. It is widely accepted that pH changes (as well as increases in osmolality) may alter the mutant frequency. However, it was noted that the resulting top dose level was much lower than in the study by Jensen (1991, TOX9552372) who did not report a decline in pH at concentrations above 1 mg/mL. This obvious difference might suggest some variability in the

acidic properties of the test materials although it seems not entirely clear from the study report if Jensen (1991, TOX9552372) had in fact measured the pH after treatment.

B.6.4.3 Tests for DNA damage and repair in mammalian cells and bacteria

A direct interaction of glyphosate acid with DNA, *i.e.*, DNA damage and repair was investigated in a small number of studies in different test systems *in vitro*. A UDS assay in primary rat hepatocytes by Rossberger (1994, TOX9400697) was reported in detail in the previous DAR (1998, ASB2010-10302) that is still considered acceptable. In contrast, the other studies in which this endpoint was addressed by different test methods and that had been provided for the previous EU evaluation must be considered not acceptable from a today's point of view. Thus, the UDS assay by Williams (1983, TOX9552370), a DNA repair assay with the IPA salt in *E. coli* by Wang (1993, TOX9500380), the SCE assays by Wang *et al.* (1993, TOX9500381, again, IPA salt tested) and by Jenkinson (1990, TOX9500269) and a Rec-assay by Shirasu *et al.* (1980, TOX9552408) were excluded from current re-evaluation of glyphosate. In contrast, a more recent rec assay in *Bacillus subtilis* has been submitted and is described in detail and evaluated by the RMS below Table B.6.4-6.

Table B.6.4-6: Summary of valid *in vitro* tests with glyphosate acid for DNA damage and repair in mammalian cells and bacteria (provided by the Notifiers)

Reference; study identification; owner		Type of study	Test organism / test system	Dose levels; batch/lot no.; purity	Results
<i>In vitro</i> tests for DNA damage and repair in mammalian cells					
Previously known	Rossberger, 1994; TOX9400697; Feinchemie	UDS assay	Primary rat (Sprague-Dawley) hepatocytes	0.20 – 111.69 mM; F/93/032; >98%	Negative
<i>In vitro</i> tests for DNA damage and repair in bacteria					
New study	Akanuma, 1995b; ASB2012-11477; Arysta	Rec assay	<i>B. subtilis</i> strains H17 and M45 (+/- S9)	+/- S9 : 7.5 – 240 µg/disk; Lot 940908-1; 95.68%	Negative (supplementary study)

Rec assay in *B. subtilis* (Akanuma, 1995)

Reference: IIA, 5.4.3/02

Report: Akanuma, M. 1995b HR-001: DNA Repair Test (Rec-Assay).
The Institute of Environmental Toxicology, Tokyo, Japan
Data owner: Arysta LifeScience
Study No.: IET 94-0141
Date: 1995-03-14

Guidelines: U.S. EPA FIFRA Guidelines, Subdivision F

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 1995-02-14 to 1995-02-15

Materials and methods

Test material:	Glyphosate technical
Identification:	HR-001
Description:	Solid crystals
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	Not mentioned in the report
Solvent used:	Sterile water
control materials:	
Negative:	Kanamycin (without activation)
Solvent/final concentration:	Water / > 12 mg/mL
Positive: non-activation	mitomycin C (without activation)
and activation	3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (with activation)
activation:	The enzyme activity measured by mutagenicity was good. S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl ₂ , 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.
test organisms:	Recombination-wild (rec ⁺) strain H17 Recombination-deficient (recE ⁻) strain M45 of <i>Bacillus subtilis</i>
test concentrations	6 dose level were tested: 7.5, 15, 30, 60, 120 and 240 µg/disk with and without S9 metabolic activation

Study conduct

DNA-damaging activity was evaluated by a DNA repair test (Rec-Assay), with *Bacillus subtilis* strains of recombination wild (rec⁺) H17 and recombination-deficient (recE⁻) M45, at concentrations of 7.5, 15, 30, 60, 120 and 240 µg/disk with and without S9 metabolic activation. The S9 fraction for the metabolic activation was obtained from liver of male SD strain rats previously treated intraperitoneally with 30 mg/kg phenobarbital (x 1), 60 mg/kg phenobarbital (x 3) and 80 mg/kg 5,6-benzoflavone (x 1). Negative control substance, kanamycin (0.2 µg/disk) without S9, and positive control substance mitomycin C (0.01 µg/disk) without S9, and positive control substance 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (Trp-p-1, 5 µg/disk) with S9 were also tested on both strains. In addition a solvent control sterile water (20 µL/disk) without S9, and sterile water and co-factor solution (20 µL: 20 µL/disk) with S9, was included in the experiment.

Paper discs (8 mm diameter) impregnated with 20 µl of the solution of the test substance were placed on the prepared spore agar plate containing the tester organism for each test, with and without metabolic activation. Duplicate plates were used for each experimental point. Diameter of a growth inhibitory zone of each strain was measured after incubation at 37 °C for 24 hours.

Statistics

Results were judged without statistical analysis.

Evaluation Criteria

Results are judged positive when growth inhibitory zone of M45 is larger than that of H17 and the difference in diameter was 5 mm or more at one or more dose levels that caused growth inhibitory zones, or 4 mm or less in diameter in the H17 (rec⁺) strain.

Results and discussion

Analytical determinations: None

Mutations assays: HR-001 induced a growth inhibitory zone of 1 mm in diameter at the highest dose of 240 µg/disk in the (*recE*⁻) strain M45 with S9 system (Table hereafter). The differences of growth inhibitory zones between the strains H17 and M45 were 1 mm or less. On the other hand, HR-001 did not induce any growth inhibitory zone in either the (rec⁺) strain H17 with S9 system or both the strains M45 and H17 without S9 system.

The assay was considered as valid because:

- in the negative control plates treated with kanamycin, the difference of growth inhibitory zones between M45 and H17 strains was 2-3 mm
- in the positive controls of mitomycin C without S9 and Trp-p-1 with S9, growth inhibitory zone in M45 is larger than that of H17, and the difference in diameter is 19 mm and 11-12 mm, respectively.
- in the solvent control, no growth inhibitory zone was observed in either strain.

Table B.6.4-7: Results of the Rec assay

Compound	Dose (µg/disk)	S9 fraction (-)			S9 fraction (+)		
		Inhibitory zone* (mm)		Difference** (mm)	Inhibitory zone* (mm)		Difference** (mm)
		M45	H17		M45	H17	
Solvent control (H ₂ O)		0	0	0	0	0	0
		0	0	0	0	0	0
HR-001	7.5	0	0	0	0	0	0
		0	0	0	0	0	0
	15	0	0	0	0	0	0
		0	0	0	0	0	0
	30	0	0	0	0	0	0
		0	0	0	0	0	0
	60	0	0	0	0	0	0
		0	0	0	0	0	0
	120	0	0	0	0	0	0
		0	0	0	0	0	0
	240	1	0	1	0	0	0
		0	0	0	0	0	0
Negative control (Kanamycin)	0.2	8	6	2			
		9	6	3			
Positive control (Mitomycin C)	0.01	20	1	19			
		20	1	19			
Positive control (Trp-p-1)	5				11	0	11
					12	0	12

* Diameter of growth inhibitory zone subtracted the diameter of disk (8 mm)

** Diameter of growth inhibitory zone in M45 strain subtracted that in H17 strain

Conclusion by the Notifiers

Under the conditions used in this experiment, HR-001 did not have DNA-damaging activity in the bacteria.

RMS comments:

The study is considered to provide supplementary information only because the Rec assay is not a standard method for this endpoint (DNA damage and repair). Furthermore, dose selection was not explained. However, the study results are valid and it is agreed that the test compound glyphosate proved negative in this experiment, both with and without metabolic activation. This conclusion can be drawn because the evaluation criteria for a positive response were not met. The difference of growth inhibiting zone between the two strains was 1 mm at the highest concentrations and differences were 0 at lower dose levels. Thus, they were below the value obtained for the negative control (kanamycin).

When the study description in the dossier was compared to the original study report, it was noted that the study director was Mie Akanuma. Erroneously, the first name had been mentioned in the dossier instead of the authors surname.

B.6.4.4 In vitro genotoxicity testing – Tests for clastogenicity in mammalian cells

As mentioned in the 1998 DAR (ASB2010-10302), a clastogenic potential of glyphosate was mainly investigated *in vivo* and only two *in vitro* studies had been submitted for the previous evaluation of which one (György *et al.*, 1989, TOX9650157) was considered not acceptable upon re-evaluation. In contrast, a study by Van de Waart (1995, TOX9651525) is of sufficient quality and may still be used to address this endpoint. It is considered now supplementary since the dose levels were rather low if compared to the 3 further studies that were provided for this new evaluation of glyphosate. These studies are reported in detail below Table B.6.4-8. Therefore, methodical details are not given in this table.

Table B.6.4-8: Summary of *in vitro* tests for chromosome aberrations with glyphosate acid

Reference (Owner)	Type of study	Test organism / test system	Dose levels*; batch/lot number; purity	Results
Study from the 2001 evaluation Van de Waart, 1995; TOX9651525; Agrichem	Cytogenicity	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; Lot 22021; 96%	Negative (supplementary study)
Studies not reviewed in the 2001 evaluation Kyomu, 1995; ASB2012-11475; Arysta	Cytogenicity	Chinese hamster lung (CHL) cells	- S9: 62.5 – 500 µg/mL, + S9: 255 – 1000 µg/mL; Lot 949908-1; 95.68%	Negative
	Cytogenicity	CHL cells	+/- S9: 312.5 - 1250 µg/mL; Lot H95D161A; 95.3%	Negative
	Cytogenicity	Human lymphocytes	- S9: 100 – 1250 µg/mL + S9: 100 – 1250 µg/mL; P24, 95.6%	Negative

* Mostly, higher concentrations were included but these were the dose levels up to which metaphases were analysed.

1st new clastogenicity study *in vitro* (Kyomu, 1995)

Reference: IIA, 5.4.2/01
Report: Kyomu, M. 1995 HR-001: In vitro cytogenicity test.
The Institute of Environmental Toxicology, Tokyo, Japan
Data owner: Arysta LifeScience
Study No.: IET 94-0143
Date: 1995-05-29
not published, ASB2012-11475
Guidelines: U.S. EPA FIFRA Guidelines, Subdivision F
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1995-03-13 to 1995-05-09

Materials and methods

Test Material: Glyphosate technical
Description: HR-001
Lot/Batch #: 940908-1
Purity: 95.68 %
Stability of test compound: Not mentioned in the report
Solvent used: Hank's balanced salt solution and culture medium

Control Materials
Test solvent control: Hank's balance salt solution
Positive solvent control: Physiological saline (without metabolic activation)
Benzo(a)pyrene (with metabolic activation)
Positive control: Mitomycin (without metabolic activation)
DMSO (with metabolic activation)

Activation

The enzyme activity measured by mutagenicity was good.
S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor.
The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl₂, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.

Test organisms: CHL cells established from the lung of Chinese hamster

Culture medium: The growth medium was Eagle's MEM supplemented with 10 % newborn calf serum

Test concentrations:

Preliminary cytotoxicity test: 8 doses: up to 1000 µg/L for the 24-hr treatment
8 doses: up to 2000 µg/L for the 48-hr treatment

Metaphase analysis: 4 doses: 125, 250, 500 and 1000 µg/mL for the 24-hr treatment

4 doses: 62.5, 125, 250 and 500 µg/mL for the 48-hr treatment

Replicates:

Preliminary cytotoxicity test: Duplicate

Metaphase analysis: Duplicate

TEST PERFORMANCE

In life dates 1995-03-13 to 1995-05-09

Preliminary cytotoxicity test

CHL cells were seeded at a density of 1.0×10^5 cells with 5 mL of medium and incubated for 48 hours. In the direct method, the cultures were treated with HR-001 with the doses mentioned above during 24 and 48 hours. After the treatment, relative cell growth value of each culture was measured by comparing with the staining density in the concurrent solvent control.

In the metabolic activation method, the medium was replaced with 3 mL of medium containing S9 mix and then test substance was added to the cultures.

The second growth inhibition test with the activation system was carried out with higher doses.

Duplicate cultures were used for each experimental point and their relative values were averaged.

Metaphase analysis

CHL cells were seeded at a density of 2.0×10^5 cells with 10 mL of medium and incubated for 48 hours. In the direct method, the cultures were treated with HR-001 with the doses mentioned above during 24 and 48 hours.

In the metabolic activation method, the medium was replaced with 5 mL of medium containing S9 mix and then test substance was added to the cultures.

Duplicate cultures were used for each experimental point and their relative values were averaged.

Diploid metaphase cells which possessed the typical karyotype of CHL cells and polyploid metaphase cells were analysed for a structural chromosome aberration.

The following data were recorded:

Number and frequency of polyploid cells

Number and frequency of each structural chromosome aberration

Number and frequency of metaphase cells with structural chromosome aberration

Only polyploid cell having 3 or more copies of haploid number of chromosomes was scored as a numerical chromosome aberration cell.

Statistics: The number of aberrant metaphases and polyploidy cells at each dose were statistically compared with those of corresponding solvent controls using a chi-square test.

Results and discussion

Preliminary cytotoxicity test: In the 24-hr and 48-hr treatments of the direct method, the doses of HR-001 which showed a reduction of the relative cell growth by 50 % or more were 1000

and 500 µg/mL, respectively. Therefore, the doses of 1000 and 500 µg/mL were chosen as the highest doses in the 24-hr and 48-hr treatments of the cytogenetics test, respectively.

In the metabolic activation method, no cell growth inhibition effect was observed at the dose of 1000 µg/mL or less, but at 2000 µg/mL or more, their cell growth was inhibited over 50%. Therefore, the dose of 2000 µg/mL was determined to be used as the highest dose in the cytogenetics test with the metabolic activation system. It was noticed that in the both methods the color of the culture medium was turned to yellow at 500 µg/mL or more, indicating a decline of pH.

Table B.6.4-9: Preliminary growth inhibition test

Concentration (µg/ml)	Relative cell growth (%)			
	Direct method 24 h	Direct method 48 h	Metabolic activation method 6-18 h (1 st exp.)	Metabolic activation 6-18 h (2 nd exp.)
Solvent control (Hanks)	100	100	100	100
3.9	100	100	100	-
7.8	97	101	99	-
15.6	100	101	108	-
31.3	96	110	104	-
62.5	100	106	97	-
125	102	99	103	-
250	92	82	106	-
500	74	46	112	-
1000	22	9	106	100
2000	-	-	-	22
3000	-	-	-	38
4000	-	-	-	27
5000	-	-	-	25

Metaphase analysis

It was noticed that in the both methods the color of the culture medium was turned to yellow at 500 µg/ml and above, indicating a decline of pH.

In the 24-hr and 48-hr treatments, the frequencies of the aberrant metaphases (excluding gaps) were 0.5 % and 0.0 % to 1.0 %, respectively, showing no significant increases when compared with the concurrent solvent control. The dose of 1000 µg/mL in the 48-hr treatment gave high cytotoxicity to the cells so that chromosome preparations were not obtained from the cultures treated at this dose. There was no increase in the frequencies of polyploid metaphases at any doses of HR-001 in both 24-hr and 48-hr treatments (Table B.6.4-10).

On the other hand, MMC used as a positive control caused a great increase in a frequency of aberrant metaphases that was consistent with the historical control data.

In the presence of a metabolic activation system, the frequencies of the aberrant metaphases (excluding gaps) were in the range of 0.5 % to 1.0 %, showing no significant increases when compared with the concurrent solvent control and with the solvent control, respectively. The dose of 2000 µg/ml gave high cytotoxicity to the cells so that chromosome preparations were not obtained from the cultures treated at this dose. The frequencies of polyploid cells did not significantly increase (Table B.6.4-11). The same holds true when the test compound was tested under identical conditions in the absence of metabolic activation.

On the other hand, B(a)P used as a positive control caused a remarkable increase in the frequency of aberrant metaphases in the presence of S9 mix that was consistent with the historical control data.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Table B.6.4-10: Cytogenetics test (direct method, i.e., without metabolic activation, 24-hr and 48-hr treatments)

Treatment	Time (h)	S9 Mix	Dose (µg/ml)	Number of metaphase	Mitotic index (%)	Polyploid		Number of chromosome aberrations								Number of aberrant metaphases		
							Judge	Gap g	ctb	Cte	csb	cse	Fragmen-tation	Others	+g	-g	Judge	
Untreated control	24	-	0	100	6.1	0	-	1.0	0	0	0.5	0	0	0	1.5	0.5	-	
	48	-	0	100	2.5	0.5	-	0.5	1.0	0	0	0	0	0	1.5	1.0	-	
Solvent control (Hanks)	24	-	10%	100	6.0	0.5		0	0	0	0	0	0	0	0	0		
	48	-	10%	100	3.4	0.5		0	0	0	0	0	0	0	0	0		
HR-001	24	-	125	100	6.7	0.5	-	0	0	0	0	0.5	0	0	0.5	0.5	-	
			250	100	5.9	0.5		1.0	0.5	0	0	0	0	1.5	0.5	-		
			500	100	5.6	0		1.5	0.5	0	0	0	0	2.0	0.5	-		
			1000															
	48	-	62.5	100	3.0	0	-	1.5	0	0	0	0	0	0	1.5	0	-	
			125	100	2.9	0	-	0.5	0.5	0	0	0	0	1.0	0.5	-		
			250	100	3.1	1.0	-	1.5	0.5	0	0	0.5	0	2.5	1.0	-		
			500	100	3.0	0	-	1.5	1.0	0	0	0	0	2.5	1.0	-		
Positive control (MMC)	24	-	0.1	100	3.3	0.5	-	4.0	21.5	39.5	2.5	1.0	0	0.5	50.5	49.0	+	
	48	-	0.1	100	2.6	1.0	-	5.5	43.0	49.5	2.5	3.0	1.5	1.5	72.0	70.5	+	

Ctb: chromatide break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; +g: including gaps; -g: excluding gaps; MMC: mitomycin C

Table B.6.4-11: Cytogenetics test (with metabolic activation, 6-hr treatment, followed by fixation for 18 hours)

Treat-ment	Time (h)	S9 Mix	Dose (µg/ml)	Number of metaphase	Mitotic index (%)	Polyploid		Number of chromosome aberrations								Number of aberrant metaphases		
							Judge	Gap g	Ctb	Cte	csb	cse	Fragmentation	Others	+g	-g	Judge	
Untreated control	6	+	0	100	4.9	0	-	1.5	0.5	0	0	0	0	0	2.0	0.5	-	
		-	0	100	5.3	0	-	0.5	1.0	0.5	0	0	0	0	2.0	1.5	-	
Solvent control (Hanks)	6	+	10%	100	6.3	0	-	1.5	0.5	0	0	0	0	0	1.5	0.5	-	
		-	10%	100	5.7	1.0	-	1.0	0	0	0	0	0	0	1.0	0	-	
HR-001	6	+	250	100	6.7	0	-	0	1.0	0	0	0	0	0	1.0	1.0	-	
			500	100	5.6	0	-	1.0	1.0	0	0	0	0	0	2.0	1.0	-	
			1000	100	7.2	0	-	1.0	0.5	0	0	0	0	0	1.5	0.5	-	
			2000															
		-	250	100	5.1	0	-	1.5	1.0	0	0	0	0	0	2.0	1.0	-	
			500	100	4.9	0	-	0.5	0.5	0	0	0	0	0	1.0	0.5	-	
			1000	100	5.7	0.5	-	0.5	0.5	0	0	0	0	0	1.0	0.5	-	
			2000															
Positive control (B(a)P)	6	+	40	100	3.0	0	-	3.0	21.0	30.0	2.0	0.5	0	0	39.5	38.5	+	
		-	40	100	4.7	0.5	-	0	0.5	0	0	0	0	0	0.5	0.5	-	

Ctb: chromatide break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; +g: including gaps; -g: excluding gaps; B(a)P: benzo (a) pyrene

Conclusion by the Notifiers

In the direct and metabolic activation system, there was not a significant increase in the frequencies of abnormal metaphases with structural chromosome aberrations or polyploid metaphases. Based on the results obtained, it was concluded that, under the conditions of this study, the test substance HR-001 did not induce chromosome aberrations in Chinese hamster CHL cells with or without the metabolic activation system.

RMS comments:

The study is considered acceptable. No evidence of an increase in structural or numerical chromosome aberrations was obtained. With regard to the study description in the dossier, it must be clarified that metaphases were analysed up to a concentration of 500 µg/mL in the first series of experiments without metabolic activation (called above and in the study report "direct method") and 24- or 48-hour treatment periods. In the experiments with and without activation and an exposure period of 6 hours (thereafter, cells were fixed for 18 hours), metaphases could be analysed up to a concentration of 1000 µg/mL. At concentrations above, evaluation was avoided by severe cytotoxicity.

2nd new clastogenicity study in vitro (Wright, 1996)

Reference:	IIA, 5.4.2/02
Report:	Wright, N.P. 1996 Technical glyphosate: Chromosome aberration test in CHL cells <i>in vitro</i> Safepharm Laboratories, Derby, UK Data owner: Nufarm SPL Project No.: 434/015 Date: 1996-03-13 unpublished, ASB2012-11476
Guidelines:	not specified
Deviations:	not specified
GLP:	yes
Acceptability:	See RMS comment

Dates of experimental work: 30 August 1995 and 4 January 1996.

Materials and methods

Test material:	
Identification:	TECHNICAL GLYPHOSATE
Description:	white powder
Lot/Batch #:	H95D 161A
Purity:	95.3 % w/w
Stability of test compound:	Not specified
Vehicle/Negative Controls	suspended in minimal essential culture media

Mitomycin C (MMC, Sigma Batch No. 104H2504)
0.05 µg/mL for cultures treated for 24 or 48 hours in the
absence of metabolising enzymes.
Cyclophosphamide both with and without metabolic
activation
Lot No. Aro. S9/11/OCT/95 SPL was prepared in-house at
Safepharm Laboratories on 11/OCT/95. It was prepared
from the livers of male SpragueDawley rats weighing -
200 g. These had received a single ip. injection of Aroclor
1254 at 500 mg/kg, up to 5 days before
Test organisms:: Hamster CHL line
Eagle's Minimal Essential medium with Earle's Salts
Culture medium: (MEM), supplemented with 10 % foetal bovine serum and
antibiotics, at 37° C with 5 % CO₂ in AIR
Test concentrations:: 19.5 to 5000 µg/mL

Study conduct:

A preliminary toxicity test was performed on cell cultures using 24 and 48-hour continuous exposure times without metabolic activation and a 6-hour exposure period both with and without metabolic activation, followed by an 18-hour recovery period in treatment-free media. The dose range used was 19.5 to 5000 µg/mL. Growth inhibition was estimated by counting the number of cells at the end of the culture period on an electronic cell counter (Coulter) and expressing the cell count as a percentage of the concurrent vehicle control value. Slides were also prepared from the cells in order to check for the presence of cells in metaphase.

- Without Metabolic Activation

- i) 24 hours continuous exposure to the test material prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL
- ii) 48 hours continuous exposure to the test material prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL.

- With Metabolic Activation

- i) 6 hours exposure to the test material and 59-mix (0.5 mL per 4.5 mL culture medium of 10 % 59 in standard co-factors). A phosphate buffered saline wash and then a further 18 hours in treatment-free media prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL.
- ii) 6 hours exposure to the test material without 59-mix. A phosphate buffered saline wash and then a further 18 hours in treatment-free media prior to cell harvest. This group acts as a 'control' for group i). The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL.

After exposure cells were harvested and scored for chromosome damage.

Results and discussion

Preliminary Toxicity Test:

In all cases except 6 hours with 59, the test material induced some evidence of cell toxicity. Microscopic assessment of the slides prepared from the treatment cultures showed metaphases present up to 5000 µg/mL in the 6-hour with and without 59-mix treatment cases. The maximum dose with metaphases present was 2500 µg/mL in the 24 and 48-hour continuous exposure treatment case. However, when a pH check was performed on culture media dosed with technical glyphosate it was observed that the pH was reduced in a dose-related way. At the maximum two dose levels the pH was reduced by ~ 1 unit and this was

considered to be unacceptable because alterations in pH have been shown to cause artefactual responses. Therefore the maximum dose level selected for the main study was 1250 µg/mL.

Chromosome Aberration Test

The test material was acidic at 2500 and 5000 µg/mL. Therefore the toxicity observed in the preliminary toxicity test was not relevant, and 1250 µg/mL was selected as the maximum dose for all treatment groups. The vehicle control cultures gave values of chromosome aberrations within the expected range. All the positive control cultures except cyclophosphamide without S9 gave highly significant increases in the frequency of cells with aberrations indicating that metabolic activation system was satisfactory and that the test method itself was operating as expected. The test material did not induce a statistically significant increase in the frequency of cells with aberrations at any dose level in any treatment group. The test material did not induce a significant increase in the numbers of polyploid cells at any dose level in any of the four treatment cases.

Conclusion by the Notifiers:

Technical glyphosate did not induce any statistically significant, dose-related increases in the frequency of cells with chromosome aberrations either in the presence or absence of a liver enzyme metabolising system or after various exposure times. Technical glyphosate is therefore considered to be non-clastogenic to CHL cells *in vitro*.

RMS comments:

The study is considered acceptable. There were no indications for more frequent occurrence of chromosome aberrations. Thus, the conclusion is agreed with.

3d new clastogenicity study in vitro (Fox, 1998)

Reference: IIA, 5.4.2/03
Report: Fox, V. 1998 Glyphosate Acid: *In Vitro* Cytogenetic Assay In Human Lymphocytes
 Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK
 Data owner: Syngenta
 Report No.: CTL/P/6050
 Date: 1998-10-29
 not published, TOX2000-1995
Guidelines: OECD 473 (1997): OPPTS 870.5375 (1998): 2000/32/EC B10 (2000)
Deviations: The stability and achieved concentration of the test substance and control substances in the vehicles used were not determined by analysis.
 This deviation from the current regulatory guideline is considered not to compromise the scientific validity of the study.
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1995-07-25 to 1998-10-29

Materials and methods

Test Material: Glyphosate acid
 Description: Technical; white solid
 Lot/Batch number: P24
 Purity: 95.6 % a.i
 CAS#: Not reported
 Stability of test compound: Confirmed by Sponsor

Control Materials:

Negative: Supplemented RPMI-1640 culture medium
 Solvent control 800 µL/mL
 (final concentration):
 Positive control: Absence of S9 mix: Mitomycin C, 0.2 µg/mL
 Presence of S9 mix: Cyclophosphamide 50 µg/mL

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

The metabolic activation system (S9-mix) used in this study was prepared as required (on each day of culture treatment) as a 1:1 mixture of S9 fraction and cofactor solution.

The cofactor solution was prepared as a single stock solution of Na₂HPO₄ (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and MgCl₂ (12 mM) in sterile double deionised water and adjusted to a final pH of 7.4.

Test cells: mammalian cells in culture

	V79 cells (Chinese hamster lung fibroblasts)
X	Human lymphocytes. Obtained on the days of culture initiation from healthy, non-smoking donors. Donor 1 was male and Donor 2 was female. Both donors had a previously established low incidence of chromosomal aberrations in their peripheral blood lymphocytes.
	Chinese hamster ovary (CHO) cells

Media: RPMI-1640 (Dutch modification)				
Properly maintained?	X	Yes		No
Periodically checked for <i>Mycoplasma</i> contamination?		Yes	n/a	No
Periodically checked for karyotype stability?		Yes	n/a	No

Test compound concentrations used

Donor 1 – 68 hours		Donor 2 – 68 hours		Donor 2 – 92 hours	
+ S9	-S9	+ S9	-S9	+ S9	-S9
100 µg/mL	100 µg/mL	100 µg/mL	100 µg/mL	1250 µg/mL	1250 µg/mL
750 µg/mL	750 µg/mL	750 µg/mL	750 µg/mL		
1250 µg/mL	1250 µg/mL	1250 µg/mL	1250 µg/mL		

Test performance

Cytogenetic assay: Duplicate human peripheral blood cultures were exposed to the solvent, test substance or positive control substances at appropriate concentrations in the following experiments:

A cytogenetic test using blood from Donor 1 in the presence and absence of S9-mix with a standard sampling time of 68 hours after culture initiation. Solvent and positive control cultures were included.

A second independent cytogenetic test using blood from Donor 2 in the presence and absence of S9-mix with a standard sampling time of 68 hours after culture initiation and a later sampling time of 92 hours after culture initiation. Solvent control cultures were included at both sampling times whereas the positive control cultures were only included at the 68 hour sampling time.

In both experiments a range of concentrations of glyphosate acid was used in order to define suitable concentrations for chromosomal aberration analysis.

The standard sampling time of 68 hours after culture initiation used in this study was based on a measured mean cell cycle time for cultured human peripheral blood lymphocytes of 13.5 hours in this Laboratory. The later sampling time was selected to be 24 hours after the standard sampling time.

Culture and treatment of blood samples: Cultures (10 mL) were established by the addition of 0.5 mL of whole blood to RPMI-1640 (Dutch modification) tissue culture medium supplemented with approximately 10 % foetal bovine serum (FBS), 1.0 IU/mL heparin, 100 IU/mL penicillin and 100 µg/mL streptomycin. The lymphocytes were stimulated to enter cell division by addition of phytohaemagglutinin (PHA; at 5 % v/v) and the cultures were maintained at approximately 37 °C for 48 hours with gentle daily mixing where possible.

Prior to treatment, the cultures were centrifuged and the culture medium was removed and replaced with fresh supplemented RPMI-1640 culture medium in the solvent and positive control cultures.

Approximately 48 hours after culture establishment, 8 mL aliquots of the test substance preparations were administered to duplicate cultures as appropriate to the experiment design. The positive control cultures were treated at a dosing volume of 50 µL/10 mL culture. In addition, 200 µL of a 1:1 mix of S9 and co-factor solution was added to each culture to be treated in the presence of S9-mix.

Cultures treated in the presence of S9-mix were treated for a period of approximately 3 hours at 37 °C, after which the culture medium was removed following centrifugation and replaced with fresh supplemented RPMI-1640 culture medium. The cultures were re-incubated at approximately 37 °C for the remainder of the 68 hour growth period. Cultures treated in the absence of S9-mix were maintained at approximately 37 °C for the remainder of the 68 hour growth period. All cultures due for sampling at the later 92 hour sampling time received an additional culture medium change approximately 68 hours after culture initiation.

The effect of glyphosate acid on the pH and osmolality of the culture medium was investigated, using single cultures containing medium only, as changes in pH and increases in osmolality are well known to result in the production of chromosomal aberrations.

Culture harvesting: Approximately 2 hours prior to harvesting, the cultures were treated with colcemid at a final concentration of 0.4 µg/mL. Sixty-eight hours or 92 hours after culture establishment the cultures were centrifuged, the supernatant was removed and the cells were re-suspended in approximately 10 mL of 0.075 M KCl at room temperature for approximately 10 minutes.

Details of slide preparation: The cultures were centrifuged, the supernatant was removed and the remaining cells were fixed in freshly prepared methanol/glacial acetic acid fixative (3:1 v/v) added dropwise and made up to a volume of approximately 10 mL. The fixative was removed following centrifugation and replaced with freshly prepared fixative. This fixation process was repeated at least twice prior to slide preparation on clean, moist labelled microscope slides. The slides were air dried, stained in filtered Giemsa stain (10 % Gurr's R66 in buffered [pH 6.8] double deionised water) for 7 minutes, rinsed in water, air-dried and mounted with coverslips in DPX.

Slide analysis: Slides were examined to determine that they were of suitable quality and, where appropriate, the mitotic index was determined by examining 1000 lymphocytes per culture and calculating the percentage of cells in metaphase.

For each donor, both in the presence and absence of S9-mix, duplicate cultures treated with glyphosate acid at three concentrations were selected for chromosomal aberration analysis at the 68 hour sampling time along with the appropriate solvent and positive control cultures. In each case the highest concentration was selected on the basis of reduction in the pH of the culture medium and the suitability of the metaphase preparations for chromosomal aberration analysis. In addition, duplicate cultures from the Donor 2 treated with glyphosate acid at the highest concentration selected at the 68 hour sampling time in the presence and absence of S9-mix were selected for chromosomal aberration analysis at the 92 hour sampling time along with the appropriate solvent control cultures.

The slides were coded prior to analysis and one hundred cells in metaphase, where possible, were analysed from each selected culture for the incidence of structural chromosomal damage.

Evaluation criteria: The percentages of aberrant metaphases and the number of aberrations per cell were calculated for each treatment scored, both including and excluding cells with only gap-type aberrations.

The Fisher Exact Probability Test (one-sided) was used to evaluate statistically the percentage of metaphases showing aberrations (excluding cells with only gap-type aberrations). Data from each treatment group, in the presence and absence of S9-mix, was compared with the respective solvent control group value. The data have been interpreted as follows:

No statistically significant increase in the percentage of aberrant cells (at any concentration) above concurrent solvent control values - NEGATIVE.

A statistically significant increase in the percentage of aberrant cells above concurrent solvent control values, which falls within the laboratory solvent control range - NEGATIVE.

An increase in the percentage of aberrant cells, at least at one concentration, which is substantially greater than the laboratory historical solvent control values - POSITIVE.

A statistically significant increase in the percentage of aberrant cells which is above concurrent solvent values and which is above the historical solvent control frequencies value but below that described in (c) may require further evaluation.

Significantly increased incidence of interchanges, exchange figures or re-arrangements (where none of the above criteria are met) may require further evaluation

Results and discussion

Cytogenetic assay: Small reductions in mean mitotic activity, compared to the solvent control values, were observed in cultures (37 % - Donor 1; 33 % - Donor 2) treated with the highest concentrations of glyphosate acid selected for chromosomal aberration analysis. No reductions in mitotic activity were observed for culture treated in the presence of S9-mix and harvested at the 68 hour sampling time or cultures treated in either the presence or absence of S9-mix and harvested at the 92 hour sampling time. Cultures treated with higher concentrations of glyphosate acid were considered not to be suitable for chromosomal aberration analysis due to excessive reductions in the pH of the culture medium.

Chromosomal aberration analysis: No statistically or biologically significant increases in the percentage of aberrant cells, above the solvent control values, were recorded at the 68 hour sampling time in cultures from either Donor 1 or Donor 2 treated with glyphosate acid in either the presence or absence of S9-mix.

No statistically or biologically significant increases in the percentage of aberrant cells, above the solvent control values, were recorded at the 92 hour sampling time for cultures treated with glyphosate acid in either the presence or absence of S9-mix.

The positive control materials, mitomycin C and cyclophosphamide induced statistically and biologically significant increases in the percentage of aberrant cells, compared to the solvent control cultures.

Conclusion by the Notifiers

Glyphosate acid was not clastogenic to cultured human lymphocytes treated *in vitro* in either the presence or absence of S9-mix.

RMS comments:

The study is considered acceptable and the conclusion is agreed with. The long duration of this study is surprising but was apparently due to the fact that the in-life phase was run early in 1996 but slides were evaluated not before 1998.

B.6.4.5 In vivo genotoxicity testing (somatic cells) – Metaphase analysis in rodent bone marrow, or micronucleus test in rodents

A number of bone marrow micronucleus tests, mostly in mice, and two cytogenetic studies in mouse bone marrow have been submitted by the different notifiers to address this endpoint. An overview on these studies is given in Table B.6.4-12.

Three of the previously evaluated studies in mice (██████████ 1994, TOX9400323; ██████████, 1993, TOX9551100; ██████████ 1991, TOX9552374) and one in rats (██████████ TOX9552375) as mentioned in the 1998 DAR were still found by the RMS to be of acceptable quality. In contrast, the studies by ██████████ (1990, TOX9500255), ██████████ (1989, TOX9650159) and ██████████ (1986, TOX9551957) must be regarded as not acceptable and were not taken into account for the current re-evaluation of glyphosate.

Instead, several studies of this type, mostly performed in mice and more recently, were submitted for this re-evaluation of glyphosate by the EU. All these new studies are reported in detail below Table B.6.4-12 and each was commented by the RMS. It must be emphasised that the studies by [REDACTED] (2007, ASB2012-11480) and [REDACTED] (2008, ASB2012-11481) were considered not acceptable because the dose levels employed were much too low. Therefore, they have been deleted from the summary table.

Table B.6.4-12: Summary of valid *in vivo* micronucleus assays or cytogenic studies with glyphosate

Reference, study identification, owner		Type of study	Test organism / test system, route/ treatment	Dose levels; batch/lot, purity; sampling	Results
Studies from the 2001 evaluation	██████ 1994; TOX9400323; Feinchemie	Cytogenicity in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day; batch 046, 96.8%; sampling 24 h after second dose	Negative; mitotic index ↓ at 5000 mg/kg bw
	██████ 1993; TOX9551100; Feinchemie	Micronucleus test in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day; batch 60, 96.8%; sampling 24 h after second dose	♂: negative ♀: weakly positive at highest dose
	██████ 1991; TOX9552374; Cheminova	Micronucleus test in bone marrow	NMRI mice, single oral application	0 – 5000 mg/kg bw; 206-JaK-25-1, 98.6%; sampling after 24, 48, 72 h	Negative
	██████ 1983*; TOX9552375; Monsanto	Cytogenicity in bone marrow	Sprague-Dawley rats; single i.p. injection	0 – 1000 mg/kg bw; XHJ-64, 98.7%; sampling after 6, 12, 24 h	Negative
Studies not reviewed in the 2001 evaluation	██████ 2006; ASB2012-11478; Nufarm	Micronucleus test in bone marrow	CD-1 mice ♂; single i.p. dose	0, 150, 300, 600 mg/kg bw; H05H016A, 95.7%; sampling after 24 and 48 h	Stat. sign. ↑ in PCE at 600 mg/kg bw (24 h) but within historical control; overall: negative
	██████ 1999; ASB2012-11482; Nufarm	Micronucleus test	Swiss albino mice, ♂ + ♀, two i.p. injections (24 h interval)	0, 187.5, 375, 562.5 mg/kg bw; batch 037-919-113, 95.49 %; sampling 24 h after 2 nd application	Negative
	██████, 1996; TOX2000-1996; Syngenta	Micronucleus test in bone marrow	CD-1 mice, 5 ♂+ 5 ♀/dose / sampling point; single oral dose	0, 5000 mg/kg bw; P24, 95.6%; sampling after 24 and 48 h	Negative
	██████ 2008; ASB2012-11483; Syngenta	Micronucleus test in bone marrow	NMRI mice 6 ♂/dose/sampling point; single oral dose	0, 2000 mg/kg, sampling after 24 and 48 h, 500 & 1000 mg/kg bw sampling after 24 h only; Batch 20070545, 99.1%	Negative
	██████ 2009; ASB2012-11479; Helm	Micronucleus test in bone marrow	CD rat, single oral application	0, 500, 1000, 2000 mg/kg bw/day; batch 20080801, 98.8%; sampling after 24 and 48 h	Negative

* Results were also published by [REDACTED] 1988 (TOX9500253).

Mouse**1st new micronucleus test in mice ([REDACTED], 2006)****Reference:** IIA, 5.4.4/01**Report:** [REDACTED] 2006, Glyphosate Technical: Micronucleus Test in The Mouse
[REDACTED]

Data owner: Nufarm

Report No.: 2060/014

Date: 2006-02-08

Unpublished; ASB2012-11478

Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF**Deviations:** None**GLP:** Yes**Acceptability:** See RMS comment

Dates of experimental work: 2005-06-07 - 2005-07-20

Materials and methods

Test material:

Identification: Glyphosate Technical

Description: White crystalline solid

Lot/Batch #: H05H016A

Purity: 95.7 %

Stability of test compound: At room temperature stable until March 2008.

Vehicle and/
or positive control: PBS

Test animals:

Species: Mouse

Strain: CD-1

Source: [REDACTED]

Age: Approx. 5 - 8 weeks

Sex: Males

Weight at dosing: 21 - 29 g

Acclimation period: At least 7 days

Diet/Food: Certified Rat and Mouse Diet Code 5LF2, BCM (IPS Ltd., London UK), *ad libitum*Water: Tap water, *ad libitum*

Housing: In groups up to seven in solid-floor polypropylene cages with wood flake bedding.

Environmental conditions: Temperature: 19 - 25 °C
Humidity: 30 - 70 %
Air changes: approx. 15/hour
12 hours light/dark cycle

Animal assignment and treatment:

The test was conducted using young male CD-1 mice. Groups of seven mice each were dosed via the intraperitoneal route at 150, 300 and 600 mg/kg bw.

One group from each dose level was killed by cervical dislocation 24 hours following treatment and another group dosed with test material at 600 mg/kg bw after 48 hours. In addition, three further groups of mice were included in the study; two groups (each of seven mice) were dosed via the intraperitoneal route with the vehicle alone (PBS) and a third group (five mice) was dosed orally with the positive substance cyclophosphamide. The vehicle controls were killed 24 or 48 hours following dosing and positive control group animals were killed 24 hours following dosing.

Immediately following termination both femurs were dissected from each animal, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed in absolute methanol, stained in May-Grünwald/Giemsa, allowed to air-dry and coverslipped using mounting medium.

Results and discussion

Mortality: No mortality occurred.

Clinical observations: Clinical signs were observed in animals dosed with the test material at and above 150 mg/kg bw in both the 24 and 48-hour groups where applicable, these included as follows: hunched posture, ptosis, ataxia and lethargy.

Evaluation of bone marrow slides:

A statistically significant decrease in the percentage PCEs per 1000 erythrocytes was observed in the 24-hour 600 mg/kg bw group when compared to the control. A similar decrease was also observed in the 48-hour 600 mg/kg bw group, but the larger standard deviation resulted in no statistical significance being applied. This finding, accompanied by the presence of clinical signs, was taken to indicate that systemic absorption had occurred and exposure to the bone marrow was confirmed.

There was a small but statistically significant increase in the incidence of micronucleated PCEs in animals dosed at 600 mg/kg bw in the 24-hour group when compared to the control group. However, the response was very modest and within the historical range (see Table B.6.4-13 and Table B.6.4-14). The response seen is considered to be most likely due to a haematopoietic effect induced by the cytotoxic effect of the test material on the bone marrow rather than any genotoxic mechanism. The increased erythropoiesis caused by the test material toxicity might cause some cells to cycle more quickly than in the vehicle control animals and, therefore, there may also be less opportunity to repair spontaneously-occurring DNA damage before the final mitosis and enucleation, resulting in small increases in micronucleated cells. Therefore the response was considered to have no genotoxic significance.

The positive control group showed a marked increase in the incidence of micronucleated PCEs hence confirming the sensitivity of the system to the known clastogenic activity of cyclophosphamide under the conditions of the test.

Table B.6.4-13: Summary of results

Treatment group / sampling time	Number of PCE with micronuclei/2000 PCE		% PCE with micronuclei		% PCE / 1000 erythrocytes	
	Group mean	SD	Group mean	SD	Group mean	SD
Vehicle control (10 mL/kg) / 48h	2.0	2.4	0.10	0.12	36.01	4.39
Vehicle control (10 mL/kg) / 24h	1.3	1.1	0.06	0.06	38.46	4.58
Positive control (50 mg/kg) / 24h	60.6***	9.7	3.03***	0.49	51.46	4.45
Glyphosate (150 mg/kg) / 24 h	1.4	0.8	0.07	0.04	45.23	6.12
Glyphosate (300 mg/kg) / 24 h	1.1	1.1	0.06	0.05	38.57	8.69
Glyphosate (600 mg/kg) / 24 h	3.9*	1.5	0.19*	0.07	27.71**	4.95
Glyphosate (600 mg/kg) / 48 h	1.9	2.1	0.09	0.1	28.16	14.23

PCE = polychromatic erythrocytes

SD = standard deviation

* : p < 0.05, ** : p < 0.01, *** : p < 0.001

Table B.6.4-14: Historical control data for relative frequency categories of micronuclei per 1000 PCE*

24-h sampling			48-h sampling		
Frequency categories	Groups	%	Frequency categories	Groups	%
0.0 – 0.4	15	25	0.0 – 0.4	21	35
0.5 – 0.9	25	42	0.5 – 0.9	18	30
1.0 – 1.4	14	23	1.0 – 1.4	14	23
1.5 – 2.0	3	5	1.5 – 2.0	7	12
2.1 – 2.5	3	5	2.1 – 2.5	0	0

* Data from 60 studies

Conclusion by the Notifiers

The test material glyphosate technical was considered to be non-genotoxic under the conditions of the test.

RMS comments:

The study is considered acceptable. The selection of the highest dose level of 600 mg/kg bw is appropriate since the application route was intraperitoneal (for oral dosing, it might be too low). In fact, in a range-finding test, deaths were observed after i.p. application of 800 and 1000 mg/kg bw. The use of only male mice is also justified because males are known to be the more sensitive sex for micronucleus formation. The conclusion is supported. The increase in micronucleated PCE in high dose males after 24 hours ($3.9/2000 = 1.95/1000$) is covered by the historical controls even though it is close to its upper edge. Furthermore, no evidence of an increase was seen after 48 hours. Thus, there is no concern about clastogenicity because of this study.

2nd new micronucleus test in mice ([REDACTED], 2007)

Reference: IIA, 5.4.4/03

Report: [REDACTED] 2007 Mammalian Erythrocyte Micronucleus Test for Glifosato Técnico Helm

Data owner: HAG

Report No.: 3393/2007-3.0MN-B

Date: 2007-12-13

Unpublished; ASB2012-11480

Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 26/11/2007 – 28/11/2007

Materials and methods

Test material:

Identification: GLIFOSATO TECNICO HELM

Description: Solid,

Lot/Batch #: 2007091801

Purity: 980.1 g/kg

Stability of test compound: Stable (GPAC MT 46, 54 °C, 14 days)

Vehicle and/
or positive control: Vehicle: deionized water
Positive Control: Cyclophosphamide

Test animals:

Species: Swiss mice

Source: [REDACTED]

Age: 09 – 10 weeks

Sex: Male

Diet/Food: Commercial food (Biobase Biotec), *ad libitum*

Water: *ad libitum*

Housing: Animals were kept in groups of 6 animals in solid cages bedded with wooden chips.

Environmental conditions: Temperature: 18 - 21 °C

Humidity: ~57 %

12 hours light/dark cycle

Animal assignment and treatment:

The test was conducted using young male mice. Groups of six mice were dosed via oral route at 8.0, 15.0 and 30.0 mg/kg bw. The animals were treated twice at 0 and 24 h. Sampling was performed 24 hours after last treatment. Both femurs from each rat were dissected, aspirated

with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed and stained, allowed to air-dry and coverslipped using mounting medium. 3000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. The ratio of PCE to normochromatic Erythrocytes (NCE) was determined for each animal by counting a total of 2000 erythrocytes.

Results and discussion

Mortality: No mortality occurred.

Evaluation of bone marrow slides: When animals treated with GLIFOSATO TECHNICO HELM were compared to the concurrent negative control group, no statistically significant increase in the number of micronuclei was observed at dosage of 8 or 15 mg /kg bw (see Table B.6.4-15). At 30 mg /kg bw results obtained were statistically significant when compared to the control group but they did not have biological relevance when compared to historical control data.

Table B.6.4-15: Summary of results

Treatment group / sampling time	Number of PCE with micronuclei /18000 PCE	χ^2
Vehicle control (5 mL/kg)	11	n.a.
Positive control (75 mg/kg)	347	315.4 (p < 0.001)
Glyphosate TC (8 mg/kg)	19	2.14 (p = 0.144)
Glyphosate TC (15 mg/kg)	21	3.12 (p = 0.077)
Glyphosate TC (30 mg/kg)	25	5.44 (p = 0.020)

PCE = polychromatic erythrocytes

Conclusion by the Notifiers

The test material glyphosate technical was non-genotoxic.

RMS comments:

The study is considered not acceptable since the dose levels were much too low for any meaningful conclusion with regard to micronucleus formation. In the original report, some justification for dose selection is given, based on a range-finding test suggesting effects at rather low dose levels. These findings were obviously in contradiction to more reliable acute toxicity tests with glyphosate in the mouse. In other micronucleus assays described in this section, much higher dose levels were employed.

3d new micronucleus test in mice (Costa, 2008)

Reference:

IIA, 5.4.4/04

Report:

██████████. 2008 Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice

██

Data owner: HAG

Report No.: RF - 3996.402.395.07

Date: 2008-09-29

Unpublished; ASB2012-11481

Guidelines:

OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF

Deviations:

None

GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 19/05/2008 – 13/08/2008

Materials and methods

Test material:

Identification: GLYPHOSATE TECHNICAL
 Description: Solid,
 Lot/Batch #: 20070606
 Purity: 980.0 g/kg
 Stability of test compound: Stable to hydrolysis at pH 3, 6 and 9 (5-35 °C)

Vehicle and/ Vehicle: sterile corn oil
 or positive control: Positive Control: Cyclophosphamide

Test animals:

Species: Swiss mice
 Source: Anilab Animals, Laboratório Cricao Ltda.
 Age: 07 – 12 weeks
 Sex: Male and Female
 Weight at dosing: approx. 30 g
 Diet/Food: Commercial food (Purina Labina, Agribbrands Purina do Brsail, Ltda.)
 Water: *ad libitum*
 Housing: Animals were kept in groups of 5 animals by sex in solid cages bedded with sterile sawdust.
 Environmental conditions: Temperature: 20 - 24 °C
 Humidity: 50 - 70 %
 Air changes: approx. 10 - 15/hour
 12 hours light/dark cycle

Animal assignment and treatment:

The test was conducted using young male and female mice. Groups of five male and five female mice were dosed via oral route (positive reference item was administered via intraperitoneal route) at 15.62, 31.25 and 62.5 mg/kg bw in a final volume of 15 mL per dose. Sampling was performed after 24 hours for all groups and after 48 hours for the vehicle control and the highest dose group. Both femurs from each rat were dissected, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed and stained, allowed to air-dry and coverslipped using mounting medium. 2000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. The ratio of PCE to normochromatic Erythrocytes (NCE) was determined for each animal by counting a total of 1000 erythrocytes.

Results and discussion

Mortality: No mortality occurred.

Evaluation of bone marrow cells: The statistical analysis of the results pointed out that the test substance did not induce an increase in micronuclei number in polychromatic erythrocytes of the bone marrow when compared to the negative control at any evaluated concentrations. No adverse effect was observed in the ratio of polychromatic erythrocytes to normochromatics in animals treated with the test substance glyphosate technical, at any evaluated concentrations. A significant statistical increase of micronucleated cells in polychromatic and normochromatic erythrocytes was observed in animals treated with cyclophosphamide, as expected (see Table B.6.4-16).

Table B.6.4-16: Summary of results

Treatment group / sampling time	male		female	
	Number of PCE with micronuclei /2000 PCE, Group mean	PCEs/NCEs	Number of PCE with micronuclei /2000 PCE, Group mean	PCEs/NCEs
Vehicle control (15 mL/kg) / 24h	0.0	1.78197	0.0	1.77195
Positive control (27 mg/kg) / 24h	0.0	1.76831	0.0	1.79107
Glyphosate TC (15.62 mg/kg) / 24 h	0.0	1.74353	0.0	1.76047
Glyphosate TC (31.25 mg/kg) / 24 h	0.0	1.71071	0.0	1.78676
Glyphosate TC (62.5 mg/kg) / 24 h	23.0**	1.54855	12.2**	1.72844

PCE = polychromatic erythrocytes

SD = standard deviation

**p<=0.01

Conclusion by the Notifiers

The test material glyphosate technical was non-genotoxic.

RMS comments:

The study is considered not acceptable since the dose levels were much too low for any meaningful conclusion with regard to micronucleus formation.

4th new micronucleus test in mice [REDACTED], 1999)

Reference: HA, 5.4.4/05

Report: [REDACTED] 1999 A micronucleus study in mice for glyphosate técnico Nufarm

Data owner: Nufarm

Study No.: RF-G12.79/99

Date: 1999-12-27

unpublished, ASB2012-11482

Guidelines: Not specified. Internal SOP M 069 - Micronucleus Test

Deviations: Not applicable

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 28/October/1999

Materials and methods

Test material:

Identification: GLIFOSATE TECNICO NUFARM

Description: White powder

Lot/Batch #: 3578/99

Purity: 95 %

Stability of test compound: No data available

Vehicle and/
or positive control: Water

Test animals:

Species: Mouse

Strain: Swiss albino

Source:

Age: 7-12 weeks

Sex: Male / Female

Weight at dosing: 30.22 gg

Acclimation period: Not specified

Diet/Food: commercial pelleted diet (Labina, Purina)

Water: Tap water, ad libitum

Housing: on wood shavings, in propylene rodent cages (five of the same sex per cage) with stainless mesh lids.

Environmental conditions: Temperature: 20-24 °C
Humidity: 50-60 %
Air changes: not specified
12-hour light/dark cycle

In life dates: 28/October/1999

Animal assignment and treatment:

Three levels of the GLIFOSATE TECNICO NUFARM were tested: 187.5, 375 and 562.5 mg/kg corresponding to 25 %, 50 % and 75 % of the LD₅₀ for mice, with ten animals (five male and five female) per level. The animals were dosed twice with intraperitoneal injections in volumes of 0.45 mL for 30 g animal within a 24 hours interval, and sacrificed 24 hours after the second injection. Negative control with water and positive control with cyclophosphamide (1.66 mg/mL in physiological solution, corresponding to 25 mg/kg), were also applied with the two injections protocol. Mice were killed by cervical dislocation 24 hours after the second dosing. From the freshly killed animal both femora were removed in total. The bones were then freed from muscle, the distal epiphyseal portion was torn off by gentle traction and the proximal end of the femur was shortened with scissors until a small opening of the marrow was visible. The bone marrow cells were gently flushed out with fetal calf serum. After centrifugation at 1,000 rev./min. for 5 min., the bone marrow cells were resuspended in fetal calf serum and smeared on glass slides which were air dried overnight. The following day, the smears were fixed in ethanol 70 % for 10 min. air dried and stained for 20 min. with Eosin-Methylene Blue solution. The slides were coded and observed with a 1,000X magnification objective in a Olympus microscope. The technicians were not allowed to know the corresponding coding in the slides. For each animal 1,000 polychromatic

erythrocytes (PCEs) and 1,000 normochromatic erythrocytes (NCEs) were examined for the presence of micronuclei (MN). The relation PCEs/NCEs were determined in the first 1,000 PCEs or NCEs enumerated. Differences in the incidence per animal of MNPCEs and MNNCEs per 1000 cells and the relation PCEs/NCEs were compared using the Kruskal Wallis test for independent samples. All the tests were compared to the negative control. The criteria for a positive response was the detection of a reproducible and statistically significant ($p \leq 0.05$) positive response for at least one dose level and the increase in the number of micronuclei to be at least twice the vehicle control. The test is considered valid only if the number of micronuclei in the vehicle control stays within the historic value of the laboratory.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No systemic or local signs of toxicity were observed during the study period.

Body weight: No significant changes observed.

Necropsy: No macroscopic changes of significance were noted.

Bone marrow evaluations

Table B.6.4-17: Summary of results

Group	Micronuclei in 1000		Polychromatic Erythr.	Normochrom. Erythr.	Polychr./Norm Ratio
	Polychr. Erythrocyte	Normochr. Erythr.			
Vehicle	0.6	0	879	997.7	0.9151
187.5 mg/Kg	0.3	0.1	879.2	978.1	0.81341
375 mg/Kg	0.6	0.3	871.7	948.4	0.9348
562.5 mg/Kg	0.5	0.3	832.8	987.8	0.8513
Cyclophosphamide	4.8*	2.0*	648.9	1029.5	0.6296

Differs statistically from the vehicle control by the Kruskal Wallis test

* $p = 0.05$, and ** for $p = 0.01$.

Conclusion by the Notifiers

Presence of micronuclei in PCEs and NCEs were similar to control animals. Animals treated with the positive control cyclophosphamide showed a significant increase in micronuclei. Therefore, under the test conditions, the GLIFOSATE TECNICO NUFARM did not have mutagenic activity in mice.

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

RMS comments:

The study is considered acceptable and the conclusion is agreed with. The real dates for the experimental phase were from 28 October to 10 December 1999.

4th new micronucleus test in mice ([REDACTED] 1996)**Reference:** IIA, 5.4.4/06**Report:** [REDACTED] 1996 Glyphosate Acid: Mouse Bone Marrow Micronucleus Test[REDACTED]
Data owner: Syngenta

Report No.: [REDACTED]/P/4954

Date: 1996-03-21

not published, TOX2000-1996

Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000)**Deviations:** The stability, homogeneity and achieved concentration of the test and control substances in the vehicles used were not determined by analysis. The certified purity and stability of the control substances are not available.

The above deviations from the current regulatory guideline are considered not to compromise the scientific validity of the study

GLP: yes**Acceptability:** See RMS comment

Dates of experimental work: 1995-10-04 to 1996-03-21

Materials and methods

Test Material: Glyphosate acid
 Description: Technical, white solid
 Lot/Batch number: P24
 Purity: 95.6 % w/w a.i.
 CAS#: Not reported
 Stability of test compound: Confirmed for the duration of the study.

Control Materials:

Negative control (if not vehicle) :	N/A	Final Volume: N/A	Route: N/A
--	-----	-------------------	------------

Vehicle:	Physiological saline	Final Volume: 20mL/kg	Route: oral
----------	----------------------	-----------------------	-------------

Positive control :	Cyclo-phosphamide	Final Doses: 65 mg/kg	Route: oral
--------------------	-------------------	-----------------------	-------------

Test Animals:

Species: Mouse

Strain: CD-1

Age/weight at dosing: 6-7 weeks / 22.8-37.6 g

Source: [REDACTED]

Housing: Up to 5/cage

Acclimatisation period: At least 5 days

Diet: CT1 (supplied by Special Diets Services, Stepfield, Witham, Essex, UK *ad libitum*)Water: Mains water *ad libitum*

Test Animals:

Environmental conditions Temperature: 19-23 °C
 Humidity: 40-70 %
 Air changes: 15/hour
 Photoperiod: 12hours dark/12 hours light

Test compound administration:

	Dose Levels	Final Volume	Route
Preliminary:	5000 mg/kg	20 mL/kg	oral
Main Study:	5000 mg/kg	20 mL/kg	oral

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethality or severe toxicity observed over a four-day observation period following a single oral dose of 5000 mg/kg.

Micronucleus Test: Male and female mice were weighed and given a single oral dose and sacrificed 24 or 48 hours after dosing as shown in the table below:

Table B.6.4-18: Experimental Design

Treatment	Dose	Number of Animals /Time of kill	
		24 hours	48 hours
Glyphosate acid	5000 mg/kg	5 male and 5 female	5 male and 5 female
Vehicle control	10 mL/kg	5 male and 5 female	5 male and 5 female
Positive control (cyclophosphamide)	65 mg/kg	5 male and 5 female	

Slide Preparation: All animals were killed by over-exposure to halothane followed by cervical dislocation.

Femurs were removed and stripped clean of muscle. The iliac end of the femur was removed and a fine paint brush was rinsed in saline, wiped to remove the excess and wetted with a solution of albumin (6 % w/v in physiological saline). This was then dipped into the marrow canal and two smears were painted on an appropriately labelled clean, dry microscope slide. This procedure was repeated to give four smears of marrow per slide.

The slides were allowed to air dry and were stained with polychrome methylene blue and eosin using an automatic staining machine.

Slide Analyses: Slides were coded and scored blind. Two thousand immature polychromatic erythrocytes were examined for the presence of micronuclei for each animal. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of immature to mature erythrocytes in a sample of 1000 erythrocytes.

Statistics: The incidence of micronucleated polychromatic erythrocytes and percentage polychromatic erythrocytes in the erythrocyte sample, were considered by analysis of variance at 24 and 48 hours, separately for males and females.

Analyses were carried out using the GLM procedure in *SAS* (1989). Each treatment group mean was compared with the control group mean at the corresponding sampling time using a one-sided Student's t-test, based on the error mean square in the analysis.

Results and discussion

Preliminary toxicity assay: As no clinical signs or lethalties were observed over a four day observation period, at the limit dose level of 5000 mg/kg, this was selected to represent the maximum tolerated dose for both males and females.

Micronucleus test: No adverse reactions to treatment were observed for either males or females dosed with glyphosate acid at the limit dose of 5000 mg/kg.

No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed in either males or females at either sampling time investigated.

No statistically significant differences in the percentage of polychromatic erythrocytes, between the vehicle control and glyphosate acid treated animals, were observed in either males or females at either sampling time investigated.

The test system positive control, cyclophosphamide, induced statistically and biologically significant increases in the frequency of micronucleated polychromatic erythrocytes in both male and female mice at the 24 hour sampling time.

Conclusion by the Notifiers

Glyphosate acid, under the conditions of test, was not clastogenic in the mouse micronucleus test.

RMS comments:

The study is considered acceptable and the conclusion is agreed with.

5th new micronucleus test in mice [REDACTED] (2008)

Reference: IIA, 5.4.4/07

Report: [REDACTED]. 2008 Glyphosate Technical – Micronucleus Assay in Bone Marrow Cells of the Mouse

[REDACTED]
Data owner: Syngenta

Report No.: 1158500

Date: 2008-06-09

not published, ASB2012-11483

Guidelines: OECD 474 (1997); EPA OPPTS 870.5395 (1998); 2000/32/EEC B.12 (2000)

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 2008-02-11 to 2008-06-09

Materials and methods

Test Material: Glyphosate technical
 Description: solid, white
 Lot/Batch number: 20070545
 Purity: 99.1 % w/w glyphosate (estimated error \pm 0.3 %)
 CAS#: 1071-83-6
 Stability of test compound: not available

Control Materials:			
Negative control (if not vehicle) :	N/A	Final Volume: N/A	Route: N/A
Vehicle:	0.5 % CMC	Final Volume: 20 mL/kg	Route: oral
Positive control :	Cyclophosphamide	Final Doses: 40 mg/kg	Route: oral

Test Animals:
 Species: mouse
 Strain: NMRI
 Age/weight at dosing: 7-8 weeks
 Source: XXXXXXXXXX
 Housing: 1/cage
 Acclimatisation period: At least 5 days
 Diet: *ad libitum*
 Water: tap water *ad libitum*
 Environmental conditions: Temperature: 19-25 °C
 Humidity: 30-70 %
 Air changes: 15/hour
 Photoperiod: 12hours dark/12 hours light

Test compound administration:			
	Dose Levels	Final Volume	Route
Preliminary:	2000 mg/kg b.w.	20 mL/kg b.w.	oral
Main Study:	500, 1000, 2000 mg/kg b.w.	20 mL/kg b.w.	oral

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethalties or severe toxicity observed over a two-day observation period following a single oral dose.

Micronucleus Test:

Table B.6.4-19: Experimental Design

Treatment	Dose	Number of Animals /Time of kill	
		24 hours	48 hours
Vehicle control	10 ml/kg	6*	6*
Positive control	40 mg/kg	6*	
Test substance	2000 mg/kg	6*	6*
Test substance	1000 mg/kg	6*	
Test substance	500 mg/kg	6*	

*: the 6th animal was used as a reserve.

Slide Preparation: All animals designated for bone marrow smears were killed by over-exposure to CO₂ followed by bleeding.

The animals were sacrificed using CO₂ followed by bleeding. The femora were removed, the epiphyses were cut off and the marrow was flushed out with foetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald (Merck, D-64293 Darmstadt)/Giemsa (Merck, D-64293 Darmstadt). Cover slips were mounted with EUKITT (Kindler, D-79110 Freiburg). At least one slide was made from each bone marrow sample.

Slide Analysis: Slides were coded and scored blind. Two thousand immature erythrocytes were examined for the presence of micronuclei for each animal. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of immature to mature erythrocytes and expressed in immature erythrocytes per 2000 erythrocytes.

Results and discussion

Preliminary toxicity assay: In a pre-experiment 4 animals (2 males, 2 females) received orally a single dose of 2000 mg/kg b.w. glyphosate technical formulated in 0.5 % CMC. The volume administered was 20 mL/kg b.w..

The animals treated with 2000 mg/kg b.w. did not express any toxic reactions.

Micronucleus test: In the main experiment for the highest dose group 12 males received orally a single dose of 2000 mg/kg b.w. glyphosate technical formulated in 0.5 % CMC. For the mid and low doses 6 males per group received orally a single dose of 1000 or 500 mg/kg b.w. Glyphosate Technical formulated in 0.5 % CMC. The volume administered was 20 mL/kg b.w.

Neither the test item treated animals nor those treated with the vehicle control (0.5 % CMC) expressed any toxic reactions.

The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control, indicating that glyphosate technical did not have any cytotoxic properties in the bone marrow.

In comparison to the corresponding vehicle controls there was no biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after treatment with Glyphosate Technical were near to the value of the vehicle control group and within the historical vehicle control range.

Conclusion by the Notifiers

In conclusion, it can be stated that during the study described and under the experimental conditions reported, the test item did not induce micronuclei as determined by the micronucleus test in the bone marrow cells of the mouse.

RMS comments:

The study is considered acceptable and the conclusion is agreed with. It seems that technical material from a different (Chinese) source and of rather high purity was tested.

Rat**1st new micronucleus test in rats [REDACTED], 2009)****Reference:** IIA, 5.4.4/02**Report:** [REDACTED] 2009 Micronucleus test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration
[REDACTED]

Data owner: HAG

Report No.: [REDACTED] 23917

Date: 2009-05-18

Unpublished; ASB2012-11479

Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF**Deviations:** None**GLP:** Yes**Acceptability:** See RMS comment

Dates of experimental work: 04/02/2009 – 06/03/2009

Materials and methods

Test material:

Identification: Glyphosate TC

Description: Solid, white powder

Lot/Batch #: 20080801

Purity: 988.0 g/kg

Stability of test compound: Stable for two years at ambient temperature

Vehicle and/
or positive control: Vehicle: 0.8 % hydroxypropylmethylcellulose
Positive Control: Cyclophosphamide

Test animals:

Species: Rat

Strain: CD

Source: [REDACTED]

Age: Males: 32 – 33 days
Females: 33 – 34 days

Sex: Male and Female

Weight at dosing: Males: 106 – 132 g
Females: 88 – 111 gDiet/Food: Commercial ssniff[®] R/M-H V1534, feeding was discontinued approx. 16 hours before administrationWater: Tap water, *ad libitum*

Housing: Animals were kept in groups of 2 – 3 animals by sex in solid cages with wood flake bedding.

Environmental conditions: Temperature: 19 - 25 °C
Humidity: 30 - 70 %
Air changes: approx. 15/hour
12 hours light/dark cycle

Animal assignment and treatment:

The test was conducted using young male and female CD rats. Groups of five male and five female rats were dosed via oral rout (positive reference item was administered via intraperitoneal route) at 500, 1000 and 2000 mg/kg bw. Sampling was performed after 24 hours for all groups and after 48 hours for the vehicle control and the highest dose group. Both femurs from each rat were dissected, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed in absolute methanol, stained in Mayers Haemaleum and eosin, allowed to air-dry and coverslipped using mounting medium. 2000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. The ratio of PCE to normochromatic Erythrocytes (NCE) was determined for each animal by counting a total of 1000 erythrocytes.

Results and discussion

Mortality: No mortality occurred.

Clinical observations: No signs of systemic toxicity were noted after administration of glyphosate TC up to the highest reasonable dose level of 2000 mg glyphosate TC/kg b.w. until 48 hours after administration (the last sampling time point).

Evaluation of bone marrow slides:

No test item-related increase of micronucleated polychromatic erythrocytes was observed in the treated groups as compared to the corresponding vehicle control group (see Table B.6.4-20) at the two sampling times. The positive reference item group which received cyclophosphamide (27 mg/kg b.w., i.p.) exhibited a significant increase in the number of micronucleated polychromatic erythrocytes. Historical control data is shown in Table B.6.4-21.

Table B.6.4-20: Summary of results

Treatment group / sampling time	Number of PCE with micronuclei/2000 PCE (male animals)		Number of PCE with micronuclei/2000 PCE (female animals)	
	Group mean	SD	Group mean	SD
Vehicle control (20 mL/kg) / 48h	2.0	1.9	2.2	1.3
Vehicle control (20 mL/kg) / 24h	1.6	1.1	1.8	0.4
Positive control (27 mg/kg) / 24h	30.2	10.5	24.0	4.9
Glyphosate TC (500 mg/kg) / 24 h	1.0	1.2	1.2	1.3
Glyphosate TC (1000 mg/kg) / 24 h	0.8	0.4	1.6	0.9
Glyphosate TC (2000 mg/kg) / 24 h	1.2	0.8	0.8	0.8
Glyphosate TC (2000 mg/kg) / 48 h	1.6	0.9	0.8	0.8

PCE = polychromatic erythrocytes

SD = standard deviation

Table B.6.4-21: Historical control data

Sex		Group mean ratio PCE/-NCE ^{#1}	Group mean frequency of micronucleated PCE (per 1000) ^{#1}	Animals (%) with 0, 1 or more micronucleated PCE (per 1000) ^{#2}						
				0	1	2	3	4	5	>6
Males	Mean	0.87	1.97	11.3	34.7	30.0	10.7	6.7	4.0	2.7
	Range	0.26 – 2.94	0.4 – 5.7							
Females	Mean	0.76	1.86	14.0	30.0	21.3	18.7	7.3	5.3	3.3
	Range	0.32 – 1.47	0.4 – 4.7							

#1 Average of group means from the most recent background data. Data from 24, 48 and 72 hour samplings are combined.

#2 Individual animal profile based on the above experiments; data from 300 animals.

m male

f female

PCE polychromatic erythrocytes

NCE normochromatic erythrocytes

Conclusion by the Notifiers

The test material glyphosate technical was non-genotoxic.

RMS comments:

The study is considered acceptable and the conclusion is agreed with. Thus, absence of clastogenicity in vivo was also confirmed in the rat.

B.6.4.6 In vivo studies in germ cells

For the previous EU glyphosate evaluation, dominant lethal assays in rats and mice had been submitted in which genotoxic effects on germ cells had been examined in. The studies by [REDACTED] (1992, TOX9551102) in rats and by [REDACTED] (1980, TOX9552377) in mice may be still considered acceptable. No genotoxic effect of glyphosate on germinal tissues was found up to the highest dose levels. No new data became available since then.

Table B.6.4-22: In vivo germ cell genotoxicity testing of glyphosate acid in rats (provided by the Notifiers)

Reference; study identification; owner	Type of study	Test organism / test system	Dose levels; batch, purity	Results
Studies from the 2001 evaluation [REDACTED] 1992; TOX9551102 (FSG)	DLT	Wistar rats, single oral dose, 10 successive one-week mating periods (1:1 sex ratio)	0, 200, 1000, 5000 mg/kg bw/day; batch 60, 96.8 %	Negative
	DLT	CD-1 mice, single oral dose; each treated male mated with a total of 16 females over a period of 8 weeks	0, 200, 800, 2000 mg/kg bw/day; XHJ-64, 98.7 %	Negative

DLT = dominant lethal test

B.6.4.7 Genotoxicity of formulations (taking into account published data that were released before 2000)

In contrast to studies with the active ingredient that were, with one possible exception (██████ 1993, TOX9551100), negative, findings obtained with formulations were contradictory. This concern has been addressed by the RMS during previous EU evaluation yet (Addendum of 02 August 2000 to the DAR on glyphosate; ASB2013-2748). Because this information is considered relevant also for current re-evaluation, the descriptions and evaluations of studies and published information on mutagenicity of formulations from this addendum have been transferred into this RAR. Afterwards (see B.6.4.8), scientific publications are addressed that have been published since 2000, *i.e.*, after the addendum had been prepared.

Amended copy from the 2000 addendum (ASB2013-2748)

(current study identification by the RMS was amended and enumeration of tables was adjusted, two additional studies are reported)

I. Original studies

A total of eight mutagenicity studies using four different glyphosate formulations was made available to the Rapporteur by the companies Monsanto and Cheminova. For each of these formulations, an Ames test and a mouse bone marrow micronucleus test were submitted. The studies are reliable since they were performed at least to a large extent in compliance with current OECD guidelines (Guideline 471 for bacterial reverse mutation tests and Guideline 474 for mammalian erythrocyte micronucleus tests) under GLP-like conditions. They are all scientifically valid and may be used for risk assessment although the studies with the formulation *Glifos* are considered of limited value for this purpose only. Both test systems are widely accepted for mutagenicity testing of chemicals and respective data for glyphosate active ingredient are available allowing a direct comparison between the active substance and some of its formulations. Unfortunately, these data do not refer to those formulations for which acute toxicity studies have been submitted for purposes of EU re-evaluation of glyphosate.

The studies on *Rodeo*® were submitted as part of the joint dossier of Monsanto and Cheminova since this formulation is considered representative for the glyphosate IPA salt without any further chemicals contained. The six other study reports were kindly provided by Monsanto on request for purposes of this addendum and were not part of the original EU submission.

Following a short characterization of the products investigated, test conditions and results are summarized in Table B.6.4-23 (*in vitro* testing) and Table B.6.4-24 (*in vivo* studies). The individual studies are briefly listed below.

Brief description of formulations tested:

Rodeo® is a formulation containing 54 % glyphosate IPA salt and water but no surfactants. According to information obtained by Monsanto, it is especially intended for aquatic use. The studies have been performed and data submitted to facilitate the assessment of genotoxicity of the IPA salt since in most mutagenicity studies the test material was glyphosate acid.

The *Roundup*® formulation tested by Monsanto (*MON 2139*) is made of 31 % glyphosate (acid equivalents), [REDACTED] (*MON 0818*, i.e., a surfactant), and water.

The third tested Monsanto product *Direct*® (*MON 14445*) contains 72% glyphosate acid equivalents formulated as ammonium salt with also a [REDACTED] (Ethomeen T25, C20-C25 [REDACTED]) surfactant. According to the Rapporteurs database, it is the only glyphosate ammonium salt tested for mutagenicity.

The product called *Glifos* in Brazil (in Europe *Glyphos*) is a formulation of glyphosate manufactured by Cheminova. As indicated by the test facility [REDACTED], it contains the IPA salt at a concentration of 360 g/L. According to the German national registration data files, the product is made of the IPA salt, the by-product Berol 907, and water.

Overview on mutagenicity studies:

Table B.6.4-23: Genotoxicity studies on herbicidal formulations containing glyphosate - *In vitro* testing in bacteria (Ames test)

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Ames test	Rodeo® (containing IPA salt and water only)	<i>S. typhimurium</i> strains TA 98, 100, 1535, 1537	50 - 5000 µg/plate; -/+ S9	Negative; no signs of cytotoxicity	Kier et al., 1992 TOX9552373
Ames test	MON 2139 (Roundup® containing IPA salt, a [REDACTED] surfactant and water)	<i>S. typhimurium</i> strains TA 98, 100, 1535, 1537	5 - 500 µg/plate (-S9)/ 15 - 1500 µg/plate (+S9)	Negative; cytotoxic at the maximum dose levels, occasionally also at lower concentrations	Kier et al., 1992 TOX1999-239
Ames test	MON 14445t (Direct®, containing ammonium salt, a [REDACTED] surfactant and water)	<i>S. typhimurium</i> strains TA 98, 100, 1535, 1537	5 - 500 µg/plate (-S9)/ 15 - 1500 µg/plate (+S9)	Negative; cytotoxic at the maximum dose levels, occasionally also at lower concentrations	Kier et al., 1992 TOX1999-320
Ames test	Glifos formulation (IPA salt, Berol 907 and water)	<i>S. typhimurium</i> strains TA 97a, 98, 100 and 1535	1, 10, 100, 1000, 5000 µg/plate; -/+ S9	Negative; cytotoxic at the two upper concentrations	Vargas, 1996* TOX1999-884

* study of limited value for risk assessment only
In all trials, the solvent was distilled water.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992, TOX9552373): Ames/*Salmonella* mutagenicity assay of Rodeo®. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91184, Sponsor Project no. ML-91-441. Dates of experimental work: 26 November 1991 - 30 December 1991. GLP: yes (self-certification of the laboratory). A respective statement of the Quality Assurance Unit (QAU) is included. The study is considered acceptable.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992, TOX1999-239): Ames/*Salmonella* mutagenicity assay of MON 2139 (ROUNDUP® herbicide formulation).

Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91183, Project no. ML-91-440, Report no. MSL-11729. Dates of experimental work: 26 November 1991 - 06 January 1992. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992, TOX1999-320): Ames/*Salmonella* mutagenicity assay of MON 14445 (DIRECT® herbicide formulation). Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91185, Project no. ML-91-442, Report no. MSL-11731. Dates of experimental work: 26 November 1991 - 30 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Vargas, A.A.T. (1996, TOX1999-884): The *Salmonella typhimurium* reverse mutation by GLIFOS. BioAgri (Biotecnologia Agricola Ltda.), Piracicaba, Sao Paulo, Brazil on behalf of Cheminova; BioAgri Report G.1.1 - 050/96. Dates of experimental work: 12 October 1996 - 23 December 1996. GLP: No. However, a QAU statement is included. The study is considered of limited value for risk assessment only since a legal statement on GLP compliance is lacking and since there were some minor reporting deficiencies in particular regarding the negative (absolute and solvent) and positive control values.

Table B.6.4-24: Genotoxicity studies on herbicidal formulations containing glyphosate - *In vivo* experiments (micronucleus test)

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Micro-nucleus test	Rodeo® formulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-850-1700-3400 mg/kg bw; sampling after 24, 48 and 72 h	Negative for chromosome aberrations; overt toxicity (clinical signs, bw↓, death) at the upper dosages	██████████ 1992 TOX9552376
Micro-nucleus test	Roundup® formulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-140-280-555 mg/kg bw; sampling after 24, 48 and 72 h	Negative (no chromosome aberrations); toxic to mice at 555 mg/kg bw with some deaths occurring, cytotoxic to the bone marrow (PCE/NCE ratio↓ at 48-h sampling) at this top dose level	██████████ 1992 TOX1999-242
Micro-nucleus test	Direct® formulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-91-183-365 mg/kg bw; sampling after 24, 48 and 72 h	Negative for chromosome aberrations; signs of general toxicity at the top and, although less pronounced, mid dose level	██████████ 1992 TOX1999-322
Micro-nucleus test	Glifos formulation in distilled water	Swiss albino mice (m/f), two i.p. injections with 24-h interval	0-68-137-206 mg/kg bw; sampling at 24 h after the second dose	Negative. No indications of cytotoxic effects to the bone marrow. No information regarding general toxicity in the main study.	██████████ 1996* TOX1999-253

m/f male and female mice used

* study of limited value for risk assessment only

██████████ (1992, TOX9552376): Mouse micronucleus study of RODEO® herbicide formulation. Monsanto ██████████ on behalf of Monsanto; EHL study nos. 91201 (toxicity range-finding study,

not tabulated above) and 91205 (micronucleus test), Sponsor study nos. ML-91-435/ML-91-438. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

[REDACTED] (1992, TOX1999-242): Mouse micronucleus study of ROUNDUP® herbicide formulation. [REDACTED]

[REDACTED] on behalf of Monsanto; EHL study nos. 91200 (toxicity range-finding study, not tabulated above) and 91204 (micronucleus test), Sponsor study nos. ML-91-434/ML-91-437. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

[REDACTED] (1992, TOX1999-322): Mouse micronucleus study of DIRECT® herbicide formulation. [REDACTED]

[REDACTED] on behalf of Monsanto; EHL study nos. 91202 (two toxicity range-finding experiments, not tabulated above) and 91206 (micronucleus test), Sponsor study nos. ML-91-436/ML-91-439. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

[REDACTED] (1996, TOX1999-253): A micronucleus study in mice for the product GLIFOS. BioAgri (Biotecnologia Agrícola Ltda.), Piracicaba, Sao Paulo, Brazil on behalf of Cheminova; BioAgri Report G.1.2 - 060/96. Dates of experimental work: 08 October 1996 - 19 November 1996. Dose levels were chosen on the basis of a preliminary toxicity test (LD₅₀ determination) described in the study report. GLP: No. However, a QAU statement is included. The study is considered of limited value for risk assessment only since a legal statement on GLP compliance is lacking and since there was no information regarding general health effects of treatment to the animals. Therefore, it is not clear whether the highest possible dose was actually reached.

Assessment:

Four glyphosate formulations were tested for mutagenicity in the reverse mutation assay in bacteria as well as *in vivo* by means of the mouse bone marrow micronucleus test. Unequivocally, all these products proved negative in both test systems. Thus, it can be concluded that the formulations Rodeo®, Roundup® (MON 2139), Direct® and Glifos® containing either the IPA or the ammonium salt of glyphosate, alone or in combination with different surfactants, do not cause point (gene) mutations in various *Salmonella typhimurium* strains and are devoid of a clastogenic potential *in vivo*.

However, when the studies of the same type (Ames test and Micronucleus test) for the active substance and the formulations are compared, it becomes obvious that the highest concentrations or dosages to be tested were generally lower with the formulations except Rodeo®. This is apparently due to a higher degree of cytotoxicity as well as of general mammalian toxicity related to the formulations containing other ingredients than glyphosate salt and water.

To facilitate direct comparison of the Ames tests, the respective table from the monograph is reproduced here once more. (Remark, 2013: Deleted by the RMS since a few of these studies

that were used for previous EU evaluation are now considered not acceptable. The interested reader is kindly referred to Table B.6.4-1)

It is clearly to be seen that much higher concentrations of the active substance could be tested without causing significant cytotoxicity. According to the literature (Chan and Mahler 1992, TOX9551954), even concentrations up to 10,000 µg/plate have been reached. With the formulations described above, only Rodeo® which is made of glyphosate IPA salt and water could be successfully tested at such high concentrations. In contrast, strong cytotoxicity avoided meaningful evaluation of mutagenicity of the three other formulations at least at the highest of the selected concentrations and was still to be seen at much lower dose levels. Therefore, it can be assumed that cytotoxicity is due to the surfactants contained but not to glyphosate or its salts. The effects appeared more pronounced with Roundup® and Direct® than with Glifos® suggesting a particularly high cytotoxic activity [REDACTED] surfactants.

Regarding the micronucleus tests, a similar pattern becomes apparent. A number of micronucleus studies with glyphosate a.i. in mice is available. However, all these experiments were performed using the oral route. General and cytotoxicity (i.e. bone marrow effects) were confined to very high doses of 4000 or 5000 mg/kg bw corresponding to the known low acute oral toxicity of this compound. The only micronucleus test using the i.p. route (as with the formulations) was performed in rats. The highest dose of 1000 mg glyphosate a.i./kg bw did not cause clastogenicity ([REDACTED] 1983; TOX9552369, also published by [REDACTED] 1988, TOX9500253). As shown by [REDACTED] (1992, TOX9552373), the IPA salt when dissolved in water (Rodeo® formulation) can be given intraperitoneally to mice at a similar dose level (850 mg/kg bw) without causing neither toxicity nor clastogenic effects. Toxicity was confined to higher dosages (1700 and 3400 mg/kg bw) but genotoxicity was not observed. In contrast, toxicity of the other formulations containing surfactants was much higher although, again, no evidence of a clastogenic effect was obtained [REDACTED] 1992, TOX1999-242 and TOX1999-322; [REDACTED], 1996, TOX1999-253).

II. Published literature (before 2009)

During the past few years, a number of studies was published dealing with possible mutagenic effects of glyphosate formulations in different test systems. Scientific assessment of these data is very difficult for at least two reasons.

One main deficiency is the lack of precise description of the test material. Usually, source, composition and/or purity neither of the formulations nor, if tested, of the active ingredient are not stated at all or, at least, not sufficiently reported in the publications. It should be also taken into consideration that different formulations may be marketed in different countries under the same trademark, e.g. Roundup®. Further confusion comes from the fact that sometimes by-products in formulations (e.g. surfactants) were replaced by others but the name of the product was not changed. On request, data on the ingredients were submitted by the manufacturer Monsanto but even this information was not sufficient to clarify all uncertainties about the test substances. However, on the basis of the information available so far, it can be stated that the Roundup products used in the different published studies were not identical. Thus, it is questionable whether results obtained with one product will apply to others containing different non-active ingredients in different concentrations.

A second point of concern is the frequent use of less validated test systems with no proven relevance of the findings for human health risk assessment even if such systems may be well accepted to predict special environmental hazards. With regard to health effects, there are no current guidelines for these test methods and there is no actual experience how to assess positive findings in such test systems. For other test methods used, OECD guidelines do exist but the experiments were not carried out in compliance with these recommendations.

To facilitate presentation of data, it was decided to start with those experiments for which, in principle, widely agreed guidelines are available. Because of the large background database, the SCE assays were also included here. In the subsequent part of this section, investigations in test systems less frequently used for examination of plant protection products and with no guidelines existing are reported. As a result of this approach, one and the same publication may be referred to repeatedly on different sites.

It should be mentioned that in some publications also experiments are reported which were carried out with glyphosate active substance (i.e., the acid or one of its salts) being the test material. These data were not included in the monograph since the respective publications, for different reasons, were considered unacceptable for evaluation purposes (for justification, see description of experimental conditions below) in particular when the current OECD criteria for assessment of published data were applied. However, the findings are reported in this addendum since a direct comparison between active ingredient and formulation data may be of particular interest.

Although various test systems measuring different endpoints were used, it was tried to summarize the available studies in Table B.6.4-25 (see next pages) to facilitate general overview before the individual publications were discussed in greater detail below. For practical reasons, in particular to facilitate direct comparison, the studies were divided into sections according to the test systems and methods and the experiments separately tabulated.

Table B.6.4-25: Overview on published studies on mutagenicity of glyphosate, its salts and formulations

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
Ames test in <i>S.typhimurium</i> strains TA98 and TA100 (+/- S9 mix)	Roundup (48% glyphosate IPA; polyoxyethylene surfactant)	0 - 1440 µg/plate (calculated as glyphosate IPA salt)	Equivocal. Occasional increase in mutation rate but no clear dose response. Marked cytotoxicity from 360 or 720 µg/plate onwards.	Study not acceptable for evaluation purposes due to serious deficiencies. Reliable assessment avoided by cytotoxicity.	Rank et al., 1993 Z82234
Micronucleus test in mouse bone marrow; single i.p. administration; sampling after 24 or 48 h	Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA; surfactant)	0, 100, 150, 200 mg/kg bw (glyphosate IPA); 0, 133, 200 mg/kg bw (Roundup, calculated as IPA salt)	Negative. Indication of dose-related bone marrow cytotoxicity with the Roundup formulation but not with glyphosate IPA.	Supplementary study confirming previous results.	Rank et al., 1993 Z82234
Micronucleus test in mouse bone marrow; two i.p. administrations with a 24-h interval between; sampling after 6 and 24 h after the final dose	Glyphosate a.i. (99.9% pure) and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 mg/kg bw (2X150 mg/kg bw/d) for glyphosate; 0, 450 mg/kg bw (2x225 mg/kg bw/d) for Roundup	Weakly positive for glyphosate after 24 h and for Roundup at both sampling times. Some evidence of bone marrow cytotoxicity of Roundup.	Supplementary study (methodical deficiencies) revealing an increase in micronucleus frequency, data in contrast to previous results.	Bolognesi et al., 1997 Z59299
SCE assay in human lymphocytes	Glyphosate a.i. (99.9% pure) and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0 - 6 mg/ml for glyphosate; 0, 0.1, 0.33 mg/ml for Roundup	Positive for glyphosate from 1 mg/ml onwards and for Roundup at both concentrations. With Roundup, complete cytotoxicity at concentrations >0.33 mg/ml.	Insufficient data. In addition, a positive result in this assay is of equivocal biological significance against the background of more appropriate mutagenicity studies.	Bolognesi et al., 1997 Z59299
SCE assay in human lymphocytes	Roundup (not specified)	0, 250, 2500, 25000 µg/ml	Weakly positive at the low and mid dose level (for one of two donors). Cytotoxic at the high dose.	see comment above	Vigfusson and Vyse, 1980 TOX9700576 / ASB2012-12044
Alkaline elution assay for DNA single-strand breaks and formation of alkali labile sites in DNA obtained from liver and kidneys of mice following single i.p.administration	Glyphosate a.i. (99.9% pure) and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw; sampling after 4 and 24 h	Weakly positive after 4 h in both organs suggesting possible transient DNA damage.	Supplementary study (methodical deficiencies). Biological significance equivocal. Results in contrast to the negative outcome of the UDS assay. Effects might be also due to toxicity.	Bolognesi et al., 1997 Z59299

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
Investigations for oxidative damage in liver and kidney of i.p. treated mice by measuring the number of 8-OHdG (hydroxydesoxyguanosine) adducts	Glyphosate a.i. (99.9% pure) and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyl sulfate surfactant)	0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw (single i.p. administration); sampling after 4 and 24 h	Evidence of stimulation of oxidative metabolism in the liver (only glyphosate) or kidney (only Roundup) after 24 h.	Finding not indicative of mutagenicity but could indicate one possible mechanism of toxicity.	Bolognesi et al., 1997 Z59299
Measuring of DNA adducts by means of ³² P-postlabelling technique in the liver and kidney of mice following single i.p. administration	Glyphosate IPA salt and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyl sulfate surfactant)	0, 130, 270 mg/kg bw (glyphosate IPA); 0, 400, 500, 600 mg/kg bw (Roundup)	Weak dose-related increase in adducts with Roundup; no adducts seen with the IPA salt alone and in the control group.	Indication of possible DNA damage, however, biological significance of this finding equivocal. Further characterization of adducts needed. Toxicity not addressed. However, non-mutagenic toxic effects can also cause DNA adducts.	Peluso et al., 1998 TOX1999-318

Table B.6.4-25: Overview on published studies on mutagenicity of glyphosate, its salts and formulations (continued)

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
Comet assay for single-strand DNA breaks in tadpole erythrocytes	Roundup (41% glyphosate IPA; [REDACTED] surfactant)	0-1.69-6.75-27-108 mg/l water	Dose-related effect on DNA at 6.75 and 27 mg/l; completely lethal at 108 mg/l.	Impact of this formulation on tadpole DNA under environmental conditions indicated. Effect could be also due to toxicity. No relevance for human health risk evaluation.	Clements et al., 1997 Z101728
Test for lethal mutations in <i>Drosophila melanogaster</i> after treatment of larvae	Roundup (assumed to contain 41% glyphosate IPA and [REDACTED] surfactant); Pondmaster (probably made from 41% glyphosate IPA; alkyle sulfate surfactant)	Not specified but indicated to be around LC50 concentration.	Positive.	Not predictive for mutagenicity in mammals. Concentrations used were expected to exhibit high toxicity making evaluation of results very difficult.	Kale et al., 1995 Z73986
Anaphase-telophase allium test for chromosome aberrations in onion root cells	Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA; [REDACTED] surfactant)	0-720-1440-2880 µg/l (for Roundup calculated as IPA salt)	Roundup: increase in chromosome aberrations at the two upper levels indicating rather polyploidy than clastogenicity, no clear dose response Glyphosate IPA: negative	Effects in plant cells not predictive for mutagenicity in mammals. Testing a herbicide for genotoxic effects in plants generally doubtful since cytotoxicity may be expected.	Rank et al., 1993 Z82234
Chromosome aberration (CA) and Sister chromatid exchange (SCE) in human lymphocytes	Glyphosate (purity ≥ 98 %)	0-5.0-8.5-17.0-51.0 µM	Increase in CA and SCE frequency	Increase of SCE not dose related in highest dose group	Lioi et al, 1998a ASB2013-9836
CA and SCE in bovine lymphocytes	Glyphosate (purity ≥ 98 %)	0-17-85-170 µM	Increase in CA and SCE frequency	Increase of SCE not dose related in highest dose group	Lioi et al, 1998b ASB2013-9837

a.e. acid equivalents

Studies in test systems for which guidelines exist and/or much experience is available (i.e. Ames test, micronucleus test, SCE assay)

██████████ (1993, Z82234) studied the mutagenic potential of the herbicide Roundup and of glyphosate isopropylamine salt in different test systems *in vitro* as well as *in vivo*. An Ames test (plate incorporation test) was performed with Roundup only in the *Salmonella typhimurium* strains TA 98 and TA 100 with and without S-9 mix for metabolic activation. Evidence of mutagenicity was confined to the strain TA 98 under non-activation conditions as indicated by a slight but significant increase in the mean number of revertants at a concentration level of 360 µg/plate (calculated as IPA salt) which was also confirmed in the repeat experiment. With activation, however, no increase in mutation rate was seen up to this dose level. From the next higher concentration (720 µg/plate) onwards, cytotoxicity became apparent with and without metabolic activation avoiding meaningful evaluation. The study authors also reported a positive result for TA 100 in the presence of S9 mix at a concentration of 720 µg/plate but already the next lower dose of 360 µg had markedly reduced the number of revertants as compared to the control suggesting a cytotoxic effect. Furthermore, a dose response was lacking. Thus, the marked increase in mutation frequency at 720 µg/plate is not reliable. In the second experiment, this dose level was not included. Without activation, concentrations from 720 µg/plate onwards appeared cytotoxic. At lower concentrations, no increase in mutation rate was noted with strain TA 100.

In a micronucleus test in mouse bone marrow erythrocytes following single i.p. administration, Roundup as well as the IPA salt (i.e., a 1:1 mixture of glyphosate technical and isopropylamine) proved negative up to the highest dose of 200 mg/kg bw. However, with Roundup but not with the glyphosate IPA salt alone, there was evidence of bone marrow cytotoxicity at this top dose level as indicated by a significantly lower percentage of polychromatic erythrocytes.

Comment: According to the publication and to further information submitted by Monsanto, it is assumed that the Roundup formulation used was made of 48 % IPA salt, ██████████ surfactant, and water. The study design of the Ames test does not comply with current guideline requirements, e.g. the plate number scored was inconsistent throughout the study. The data obtained are so controversial that a reliable interpretation is not possible. Unfortunately, a complete confirmatory experiment was not performed since repeated testing was confined to the dose of 360 µg/plate and an additional concentration of 180 µg/plate was included. A more extensive study by ██████████ (1992, TOX1999-242, see above in section I) using four *S. typhimurium* strains including also TA 98 and TA 100 failed to elicit any indications of mutagenicity. This latter trial was conducted in compliance with OECD guideline 471 requirements and is of higher reliability, therefore. Of course, the Roundup formulations tested by Rank and her group and by ██████████ were not identical but similar since both contained only the active substance formulated as IPA salt, ██████████ surfactant, and water. The cytotoxicity of Roundup was described by both groups but the respective concentrations were different.

The design of the micronucleus test was also not in compliance with guideline requirements. A direct comparison between results obtained with the IPA salt and Roundup is not feasible since not exactly the same dose levels were used and since there was a difference in sampling time (24 and 48 h post dosing for the IPA experiment versus only at 24 h after administration of Roundup). The negative outcome of previous micronucleus studies with the IPA salt (Rodeo® formulation, ██████████ 1992, TOX9552376) and with a similar Roundup formulation in mice (██████████ 1992, TOX1999-242) was confirmed. The reported weak

bone marrow cytotoxicity occurring already after single i.p. administration of 200 mg Roundup/kg bw (amount calculated as the IPA salt to facilitate comparison) may be considered a possible formulation-related effect when the observations in other micronucleus studies (see section I) are taken into consideration.

In contrast, [REDACTED] (1997, Z59299) reported positive results from a micronucleus test in mouse bone marrow erythrocytes. Either glyphosate a.i. (declared as 99.9 % pure) or a Roundup formulation were administered to Swiss mice once daily by the i.p. route on two consecutive days. Cell samples were harvested at 6 and 24 hours following the final dose. A weak positive effect was observed at total dose levels of 300 mg/kg bw (2 x 150 mg/kg bw/day) after 24 hours for glyphosate and of 450 mg/kg bw (2 x 225 mg/kg bw/day) at both sampling times for Roundup.

Further data in this publication indicated for high purity glyphosate a significant and dose-dependent increase in SCE frequency in human lymphocyte cultures obtained from two female donors from a concentration of 1000 µg/mL onwards. For Roundup, this effect became apparent even at lower concentrations of 100 and 330 µg/mL.

Comment: The outcome of the micronucleus test with glyphosate a.i. is at least surprising since much higher doses of this compound had been tested before and did not reveal indications of clastogenicity (see section B.5.4.2.1 in the monograph). A direct comparison is not possible since the only available test using the i.p. route in which the highest dose of 1000 mg/kg bw proved negative ([REDACTED] 1983, TOX9552369) was performed in rats. The respective study by [REDACTED] (1993, Z82234, see above) was conducted in mice but the test material was glyphosate IPA salt and the dose administered was probably too low for meaningful evaluation. However, a number of well-performed micronucleus tests with oral administration to mice is available. Even when the low oral absorption rate of glyphosate (about 30%) is taken into account, the dose levels (up to 5000 mg/kg bw nominal) are much higher than those given by Bolognesi and her co-workers but no convincing evidence of a potential to cause chromosome aberrations in vivo was obtained. It should be emphasized that the increase in the incidence of micronucleated polychromatic erythrocytes as reported in this publication was rather weak only. The test was not performed according to the current OECD guideline. In particular, the number of animals used (three male mice per dose group) was too low since a group size of at least five is recommended. A dose response cannot be assessed since only one dose level was included. The basis for statistical comparison is questionable since it is not clear when the six control animals were sacrificed because only one group mean value was indicated. Due to these deficiencies, this isolated positive finding is not considered to provide sufficient evidence to contravene the previously obtained negative results regarding the active substance.

The same methodical shortcomings apply to the experiment with the Roundup formulation. The formulation tested is reported to contain 30.4 % glyphosate acid equivalents. The a.i. is formulated as the IPA salt. Alkyl sulfate surfactant (MON 8080) is also contained (source of information: Monsanto). The weak positive response is in contrast to the beforementioned GLP-like study by [REDACTED] (1992, TOX1999-242) in which Roundup® proved negative. However, these two Roundup formulations were not identical since the glyphosate concentrations were nearly the same but the surfactants contained were different making a direct comparison of the study results difficult. Little is known on mutagenicity of alkyl sulfate itself, however, MON 8080 proved negative in the Ames test but was clearly cytotoxic at relatively low concentrations (see section III of this addendum). Some evidence of bone marrow cytotoxicity was obtained with both Roundup products as indicated by a decrease in the ratio between polychromatic and normochromatic erythrocytes. Cytotoxicity could have

also an impact on chromosome aberration frequency. An overall, unequivocal conclusion from the experiment of Bolognesi and her group cannot be drawn, however an actual clastogenic response is not very likely. Even if a positive result could be confirmed, it would not be applicable to products containing other surfactants.

A higher SCE frequency is not considered to provide evidence of mutagenicity against the large number of studies in which glyphosate proved clearly negative. The two other studies of this type which have been submitted for purposes of toxicological evaluation of glyphosate (1990, TOX9500269 and 1993, TOX9500381, the latter using the IPA salt) did not reveal an increase in sister chromatid exchange frequency but, unfortunately, did not include the high concentrations as tested by the group of Bolognesi (see section B.5.4.1.3 of the monograph). Apart from general doubts about biological significance of a positive result in an SCE assay, some methodical deficiencies became obviously in this publication. For statistical reasons, the number of only two subjects to be included in the study appears too low for meaningful evaluation. Furthermore, the data from two experiments were pooled for the two donors and individual values were not given. Therefore, a possible influence of interindividual variation could not be sufficiently assessed by the reviewer. As shown below, this variation may well reach a considerable level. Again, the positive result obtained with Roundup at least might be also due to cytotoxicity of the formulation avoiding further testing at dose levels exceeding 330 µg/ml since no mitotic cells were present any more.

(1980, TOX9700576) also reported a weak but statistically significant increase in SCE frequency in human lymphocytes obtained from two donors when the cultures were exposed to Roundup (not specified) at concentrations of 250 and 2500 µg/mL. At the next higher concentration of 25000 µg/mL the test substance was absolutely cytotoxic.

Comment: The reported increase is doubtful since a dose response was seen in the cultures from one of the two donors only. Furthermore, this increase in SCE frequency over the control was weak only and the statistically increased values in the cultures provided from donor 1 were below the control value from donor 2. Furthermore, possible cytotoxicity was not addressed in this paper. Generally, the SCE assay is not accepted to provide convincing evidence of mutagenicity but is rather a screening test. For clarification, the study authors themselves recommended further mutagenicity tests to be conducted.

(1998a, ASB2013-9836 and 1998b, ASB2013-9837) reported an increase in CA and SCE frequency in human lymphocytes of 3 donors in concentrations between 5 and 51 µM and in bovine lymphocytes between 17 and 170 µM.

Comment: The results are questionable because a number of well performed and validated studies in vitro in mammalian cells and in vivo in mammals did not register comparable effects even in dose levels more than 10 times higher than the doses used in the studies described by (1998a and 1998b, ASB2013-9836 and 1998b, ASB2013-9837). A replication would be needed to confirm such aberrant results.

Other test systems (Comet assay in tadpole erythrocytes, tests for DNA adducts in rats and mice, *Drosophila melanogaster*, plant cells)

Clements et al. (1997, Z101728) investigated the genotoxicity of selected herbicides in *Rana catesbeiana* (bullfrog) tadpoles using the single-cell gel DNA electrophoresis test ('Comet' assay). After a previous study had shown a higher amount of DNA damage in bullfrog tadpoles inhabiting small bodies of water in agricultural areas as compared to non-agricultural

regions, the impact of Roundup and some other commonly used herbicides on the DNA of tadpole erythrocytes was investigated in this test system under alkaline conditions. This modification allows the detection of single-stranded DNA breaks which are indicated by an increase in length:width ratio of the DNA mass following electrophoresis. DNA was obtained from tadpole erythrocytes (nucleated cells in amphibians) after the animals had been exposed to different concentrations of Roundup in the surrounding water for 24 hours. Whereas the low dose of 1.69 mg/L did not cause evidence of DNA damage, a clear and dose-dependent effect became apparent at the following concentrations of 6.75 mg/L and 27 mg/L. At 27 mg/L, the effect level caused by the positive control substance methylmethanesulphonate (MMS) was already approached. The intended top dose level of 108 mg/L could not be evaluated since all tadpoles died during the exposure period. According to the study authors, the concentrations tested were well below the recommended application levels suggesting an environmental mutagenic hazard in particular for organisms living in small adjacent bodies of water that are usually the first to be affected by pesticide runoff.

Comment: Generally, information on genotoxic effects of pesticides under natural conditions is scarce and, thus, this test system may provide important information regarding environmental effects.

In this special case, however, it appears equivocal whether the observed impact on the DNA was indicative of a true mutagenic effect or rather caused by cytotoxicity. It is known that a positive response in the Comet assay may be not only the result of direct interaction with cellular DNA but can be also mediated by toxic and other effects causing apoptosis or necrosis. Cytotoxicity is not addressed in the publication because it is not directly measured in this test system. A certain degree of general toxicity can be assumed since the highest dose was completely lethal to the tadpoles. This effect could be well in line with the toxicity of certain glyphosate formulations to aquatic organisms as reported in the monograph. The Roundup product tested by Clements et al. was made of 41 % glyphosate IPA salt and MON 0818, i.e. the [REDACTED] surfactant which is already known to cause toxic effects in different test systems in vitro as well as in vivo. Of course, although there is some evidence of a cytotoxic mechanism behind the positive result in the Comet assay, a direct impact of the test compound on the DNA cannot be completely excluded.

At this time, it is not clear whether a positive result of this test obtained in tadpole erythrocytes, even if it was actually due to mutagenicity, would be of any relevance to human beings exposed. In particular, this is doubtful when the strong body of evidence that neither glyphosate nor its formulations are mutagenic as coming from many studies in various test systems is taken into consideration. Thus, the outcome of the Comet assay should be rather used for environmental hazard evaluation only. Again, the application of results obtained with one formulation to others must be critically regarded.

A possible impact on the DNA was also investigated by [REDACTED] (1997, Z59299) in further experiments. 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) or Roundup (900 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 and the formulation Roundup to stimulate oxidative metabolism in the liver (glyphosate) or in the kidney (Roundup) at 24 hours after application.

In a subsequent study from the same institute ([REDACTED] 1998, TOX1999-318), a low incidence of DNA adducts was found by means of the very sensitive ³²P-postlabeling technique in the liver and kidney of mice following single intraperitoneal administration of Roundup. All tested concentrations (400, 500 and 600 mg Roundup/kg bw, corresponding to

122, 152, and 182 mg glyphosate salt/kg bw) caused DNA adducts in both organs. A dose response was to be seen. In contrast, treatment with the vehicle (i.e., a DMSO/olive oil mixture) and with doses of 130 and 270 mg glyphosate IPA salt/kg bw did not result in DNA adduct formation.

Comment: The data from the tests for DNA damage and stimulation of oxidative metabolism (██████████ 1997, Z59299) are hardly 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. Taking into account that glyphosate proved negative in the UDS assay which is generally accepted to indicate a more frequent occurrence of DNA damage and repair (see section B.5.4.1.3 in the monograph), the published findings 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 not-mutagenic effects. Stimulation of oxidative metabolism is not a sign of mutagenicity but may elucidate a possible mechanism behind toxic effects.

The results of ██████████ (1998, TOX1999-318) and his group suggest a direct effect on the DNA. It has been shown that the observed effects were related to administration of the formulation only but not to glyphosate IPA salt. Biological significance of the results is equivocal. Generally, it is questionable whether findings after i.p. administration can be applied to more realistic exposure conditions. Of course, the occurrence of such effects also after oral intake would be much more relevant for human health evaluation. Furthermore, some deficiencies of this study make a definitive assessment difficult. It is rather equivocal what a low incidence of DNA adducts per animal as compared to no adducts in the control group actually means since a positive control substance was not included. The degree of variation between the animals is not known because only mean values for the groups comprising of 3 to 6 mice were reported and individual values are not given but would be helpful for interpretation of the results. Another point of concern is the lacking information on toxicity. At least with Roundup, one could expect marked general toxicity when the observations reported from the micronucleus tests (see section I of this addendum) and from the acute intraperitoneal toxicity studies (see section B.5.2.4 in the monograph) were taken into account. It is known that DNA adducts may be formed not only as a result of direct interaction of cellular DNA with chemicals but also occur naturally or can be even related to a treatment-dependent increase in endogenous metabolites. Thus, further characterisation of these adducts and clarification of their nature would be desirable.

██████████ (1995, Z73986) examined nine agricultural chemicals in the sex-linked recessive lethal test in *Drosophila melanogaster* for their ability to cause genotoxic damage to the germ cells leading to lethal mutations in the subsequent generations. The group of test compounds included two insecticides and seven herbicides among those were the glyphosate formulations Roundup and Pondmaster. Unlike the generally used method of feeding the test substance to adult males only, larvae were treated in this experiment. This modification was expected by the study scientists to improve the sensitivity of the test system. All products tested proved positive.

Comment: This test system is not considered predictive for mutagenicity in mammals. Generally, tests in *Drosophila* are considered helpful for screening purposes. For glyphosate, however, a large database on the basis of much more reliable test systems does exist. Furthermore, since lethal changes in spermatogonia and spermatocytes were the relevant endpoint, it appears difficult to distinguish between mutagenicity and general toxicity. The

dose level tested was not specified but it is stated in the publication that concentrations around the LC^{50} were used. At such a high dosage, some toxicity must be expected.

An anaphase-telophase allium test in onion root cells was conducted by [REDACTED] (1993, Z82234) to detect a possible induction of chromosome aberrations. The exposure period was 24 hours. In this plant system, a significant increase in the occurrence of chromosome aberrations was noted at the two upper dose levels when Roundup was tested. However, there was no dose response, since the total incidence of aberrations at 1440 µg/L was twice that seen at 2880 µg/L. The authors attributed this lack of a clear dose response to cytotoxicity, however, mitotic index was not dramatically reduced (24.2 in the mean at 2880 µg/L versus 28.2 at 1440 µg/L). According to the investigators, the type and pattern of aberrations suggest rather spindle disturbances than clastogenicity in particular when compared to the effects caused by the positive control substance MMS. In contrast, the glyphosate IPA salt did not increase the frequency of chromosome aberrations in this experiment.

Comment: The Roundup product tested was made of the IPA [REDACTED] surfactant, and water (for details see description of the Ames test and the micronucleus test portions of this study above). The more pronounced effect of the formulation as compared to the IPA salt could be explained by an improved uptake by the onion root cells as mediated by the surfactant. However, genotoxic or aneugenic effects in a plant system are generally not accepted to be indicative of mutagenicity in mammals. For glyphosate and its formulations, a number of well-performed studies in mammals is available for this purpose. Generally, it appears questionable whether a herbicide should be tested for mutagenicity in a plant cell system since at least a certain degree of cytotoxicity must be expected.

Assessment

In the whole, the published data are not sufficient to provide convincing evidence of mutagenic effects caused by glyphosate or its formulations. Of course, the effects observed in different test systems cannot be totally ignored. Looking for an explanation, the data obtained in the mutagenicity studies with formulations (see section I) must be also considered. Taking all the findings together, the effects reported in the literature appear rather due to cytotoxic properties of the formulations than to a genotoxic mode of action. The same conclusion was also reached by the Danish EPA in an assessment (Rasmussen, 1997, ASB2013-9671) which was made available to the Rapporteur. It has been already known before, that cytotoxicity is much more pronounced with glyphosate formulations than with the active substance and, therefore, is probably due to by-products or impurities. In particular, surfactants are the agents to be suspected for causing such effects.

There are even data suggesting the possibility of a direct interaction of glyphosate formulations with cellular DNA in some test systems. This is evidenced by a higher frequency of DNA adducts in mouse liver and kidneys following i.p. administration (Peluso et al., 1998, TOX1999-348) as well as from the Comet assay in tadpole erythrocytes ([REDACTED] 1997, Z104728). Since glyphosate active ingredient is apparently devoid of a DNA damaging potential (see monograph), these effects, if occurring, can be certainly assumed to be related to co-formulants. Damage to the DNA is not essentially indicative of mutagenicity but could also result from cytotoxicity. Irrespective of the origin of these effects on DNA level, they appear to be confined to very special exposure situations only and not to represent a health hazard to human beings.

III. Mutagenicity testing of surfactants

On the basis of the extensive mutagenicity database for glyphosate a.i. and formulations, the conclusion can be drawn that neither the active substance nor the formulations so far investigated are genotoxic. A certain potential of some formulations to cause damage to the DNA, however, cannot be excluded and might be related to the marked cytotoxic activity of these products. Data suggest that cytotoxicity is rather due to certain by-products used as surfactants than to the active ingredient. Thus, it is of particular interest to look at mutagenicity tests performed with such chemicals which are contained in glyphosate formulations mostly with the intention to improve the uptake of the herbicide glyphosate by the target plants. For three different surfactants, studies on mutagenicity of surfactants have been submitted. The studies are summarised in Table B.6.4-26 and briefly listed below.

Table B.6.4-26: Mutagenicity studies with surfactants contained in glyphosate formulations

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Ames test	MON 8080 dissolved in distilled water	S. typhimurium strains TA 98, 100, 1535, and 1537; plate incorporation and spot test [#] performed	0.003 - 3.0 µL/plate (+/-S9) in the plate incorporation test	Negative up to 0.9 µL/plate with and without activation; cytotoxicity occurring at this dose, complete toxicity at 3 µL/plate avoiding counting of revertants	Flowers, 1981 TOX1999-319
Ames test	MON 0818 dissolved in DMSO	S. typhimurium strains TA 98, 100, 1535, 1537; plate incorporation test with/ without metabolic activation	Lowest concentrations: 0.3 or 1 µg/plate, different maximum amounts per plate reached for the strains, i.e. TA98:300µg (-S9) 1000µg (+S9); TA100 and TA1535: 100µg (+/-S9); TA1537:100µg(-S9) 300µg (+S9)	Negative. Cytotoxic effects occurring at the maximum dose levels avoiding evaluation and occasionally also at lower concentrations. (Mutagenicity data for TA 1535 (+S9) not given probably due to excessive toxicity.)	Stegeman and Li, 1990 TOX1999-241
Ames test	Dodigen 4022 dissolved in distilled water	S. typhimurium strains TA 98, 100, 1535, 1537, 138; E.coli strain WP2uvrA; plate incorporation test	4 µg/plate - 10000 µg/plate (+/-S9)	Negative for mutagenicity. No cytotoxic effects observed.	Stammberger and Mayer, 1992 TOX1999-324
Cytogenetic study for chromosome aberrations in vitro	Dodigen 4022 dissolved in cell culture medium	Chinese hamster V79 cells	0-600-3000-6000 µg/mL; (+/-S9); 4 h exposure, sampling at 7, 18 and 28 h after start of treatment	Negative for clastogenicity and polyploidy. Reversible inhibition of cell cycle (mitotic index↓) after 7 h at the highest dose (+/- S9). Cell survival rate↓ at 3000 µg/ml and above (only without activation).	1992, TOX1999-325

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Micronucleus test	MON 0818 dissolved in corn oil	CD 1-mice (m/f), bone marrow erythrocytes	0 and 100 mg/kg bw; single i.p. injection; evaluation at 24 and 48 h after dosing	Negative. Also, no indications neither of general toxicity nor of bone marrow cytotoxicity to be observed.	[REDACTED] 1998* TOX1999-240

The spot test did not provide indications of a mutagenic response, however, does not allow quantitative assessment. This variation of the Ames test is no longer in use in routine genetic toxicology. Therefore, the data are not shown here.

* supplementary study

Flowers, L.J. (1981, TOX1999-319): Ames/*Salmonella* mutagenicity assay of MON 8080. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; Project no. ML-80-294/800281; Report no. MSL 1538. Dates of experimental work: 31 Oktober 1980 - 28 November 1980. GLP: No. When the study was performed, GLP was not compulsory. However, a Quality Assurance Audit statement is included. The study is considered acceptable.

Stegeman, S.D. and Li, A.P. (1990, TOX1999-241): Ames/*Salmonella* mutagenicity assay of MON 0818. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; Project no. EHL 89178/ML-89-461; Report no. MSL-10625. Dates of experimental work: 28 November 1989 - 29 January 1990. GLP: Yes (self-certification of the laboratory). A respective Quality Assurance Audit statement is included. The study is considered acceptable.

Stammlberger, I. and Mayer, D. (1992, TOX1999-324): Dodigen 4022: Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test) and *Escherichia coli*. Pharma Development Central Toxicology, Hoechst AG, Frankfurt/Main, Germany; Study no. 92.0336, Report no. 92.0467. Dates of experimental work: 03 June 1992 - 19 June 1992. GLP: Yes. The study is considered acceptable.

Stammlberger, I. (1992, TOX1999-325): Dodigen 4022: Chromosome aberrations *in vitro* in V79 chinese hamster cells. Pharma Development Central Toxicology, Hoechst AG, Frankfurt/Main, Germany; Study no. 92.0337, Report no. 92.1024. Dates of experimental work: 22 July 1992 - 03 November 1992. GLP: Yes. The study is considered acceptable.

[REDACTED] (1998, TOX1999-240): Mouse micronucleus screening assay of MON-0818. [REDACTED] on behalf of Monsanto; Project no. EHL 89182/ML-89-463. Dates of experimental work: 06 November 1980 - 05 February 1990. GLP: Not stated in the report. The study is considered supplementary only since it was not in compliance with OECD recommendations for tests of this type. In particular, the only dose level used was too low for definitive assessment.

Furthermore, the notifier Monsanto submitted to the Rapporteur published data suggesting that also the sorbitol ester surfactants Tween 20 and Tween 80 proved negative in either the mouse lymphoma test or in the Ames test and the mouse micronucleus test, respectively. However, since these co-formulants were not contained in the glyphosate formulations for which mutagenic effects had been reported, the respective data were not reviewed in detail.

Assessment

The available studies clearly show a lack of mutagenicity of the tested surfactants in the limited number of test systems used confirming the negative outcome of respective studies with glyphosate formulations. In contrast, marked cytotoxicity was caused in the Ames test by the [REDACTED] surfactant MON 0118 as well as by the alkyle sulfate surfactant MON 8080 suggesting that cytotoxicity observed in mutagenicity testing of formulations are mainly due to these surfactants. This assumption is supported by the result of an Ames test using the surfactant-free Rodeo® formulation ([REDACTED] 1992, TOX9552373) with no signs of cytotoxicity occurring. The more recently introduced surfactant Dodigen 4022 proved non-cytotoxic in the Ames test and caused cytotoxic effects in V79 cells at very high concentrations only.

It is widely accepted that cytotoxicity of a compound can result in positive results in mutagenicity assays and it is often difficult clearly to distinguish between true substance-related genotoxic effects and “mutagenicity” mediated by excessive cytotoxicity. A close relation between cytotoxicity and mutagenicity became apparent also in the chromosome aberration test with Dodigen 4022 ([REDACTED] 1992, TOX1999-325). The markedly reduced mitotic index at the first sampling time indicating an adverse effect at least of high doses (only the top dose concentration of 6000 µg/mL and the solvent control were assessed after 7 hours) was accompanied by a slight increase in chromosome aberration frequencies including and excluding gaps in the absence as well as in the presence of the metabolically activating S9 mix. However, at the later sampling times (18 and 28 h following substance application), the mitotic index had normalised again and there was no increase in the incidence of chromosome aberrations any more. Therefore, the test substance was considered negative in this test system. This example provides further evidence that suspected mutagenic effects of formulations as reported in section II might be readily due to cytotoxicity.

These results are in line with data suggesting a higher toxicity and irritancy of certain surfactants as compared to the active substance and to formulations as reported in chapter B.5.11 in the monograph. It can be also assumed that specific adverse effects of surfactants might have significantly contributed to the rather unexpected mammalian toxicity of some glyphosate formulations. Despite the low general toxicity of glyphosate technical, a number of poisoning incidents in humans sometimes resulting in death was reported in particular from Asian countries (see chapter B.5.9 in the monograph).

Severe intoxication was mainly characterised by a decrease in blood pressure and further cardiovascular symptoms followed by pulmonary dysfunction and renal failure and by signs of irritation in the gastrointestinal tract. Pathophysiology of poisoning is assumed to include irritation or corrosion of the intestinal mucosa resulting in electrolyte imbalances, hypovolemic shock and disturbances in the cardiovascular system. The respiratory signs, as well as renal symptoms, are considered secondary to this mechanism being caused either by pulmonary edema related to disturbed circulation or by aspiration pneumonia following emesis ([REDACTED] 1987, Z35531; see also monograph, chapter B.5.9). There is evidence that the first step, i.e. damage to gut mucosa, might be primarily caused by [REDACTED] surfactants due to their irritating properties.

Of course, the clinical reports on human poisonings with glyphosate formulations are often difficult to interpret since most of the severe intoxications were attempts of suicide. In such cases, also the frequent concomitant intake of drugs and alcohol should be considered.

However, the hypothesis of surfactant effects being involved is further supported by mechanistic and pharmacological studies (see section B.5.8.2.3 in the monograph) suggesting

that the acutely toxic effects may be caused by the [REDACTED] surfactant alone, too, and that toxicity may be even enhanced when complete Roundup formulations were tested.

Furthermore, according to the information available to the Rapporteur, the cases of severe or even fatal intoxication were related to the ingestion of glyphosate products containing [REDACTED] surfactant. Sawada and Nagai (1987, Z35531) reported two cases of human poisonings with surfactants causing clinical signs resembling very much those observed after ingestion of large amounts of Roundup.

A possible potentiation of toxicity of glyphosate IPA salt and POEA in animals was reported by [REDACTED] (1991, Z80636) who tested the acute oral toxicity of Roundup formulations in rats. Using the intratracheal route of administration being of clinical relevance in cases of aspiration, the same authors observed a marked toxic effect of Roundup and of POEA alone to the lungs but this was much less pronounced with Polysorbate-80, i.e. another non-ionic surfactant.

Mucosal irritation in the respiratory tract caused by [REDACTED] surfactant may be also behind the much lower threshold level for adverse effects of a Roundup formulation as compared to glyphosate a.i. upon subacute inhalative exposure (see section B.5.3.3.2 in the monograph, also reported by WHO/IPCS in 1994, TOX9500301).

A statement of the notifier Monsanto was submitted to the Rapporteur in October, 1998. In this paper, it is suggested that the toxic and cytotoxic effects of polyoxyethylenamine (POEA) were responsible for the observed adverse effects on health and environment. Since it is an important objective to use environmentally safe and less toxic products, the polyoxyethylen [REDACTED] surfactants were replaced at least in some Monsanto products by others. The company stated that this decision was mainly based on the eye irritation potential and the aquatic toxicity related to the formerly used substances. Accordingly, in the formulations for which toxicological data have been submitted as part of the joint dossier of Monsanto and Cheminova, surfactants of this type are not contained any more. Indeed, cytotoxicity of other surfactants, e.g. Dodigen 4022, and their potential to cause acutely toxic or irritating effects are much lower as compared to POEA.

Thus, it can be expected that the replacement of toxic and irritating surfactants like POEA by other and less critical substances may reduce the risk of death or severe health effects following intentional or accidental ingestion of glyphosate products as well as the severity of eye or respiratory tract irritation.

Recently, the notifier Monsanto provided a new assessment explaining that POEA is a group of chemicals not all capable of causing adverse effects. It is suggested that only particular substances belonging to this group might be responsible for the toxic effects described in this addendum. However, since this is clearly relevant for assessment of formulations but not for health evaluation of the active ingredient, this item should be considered on a Member state level.

B.6.4.8 Published data (released since 2000)

B.6.4.8.1 Introduction

An earlier review of the toxicity of glyphosate and the original Roundup™ formulation concluded that neither glyphosate nor the formulation pose a risk for the production of heritable/somatic mutations in humans (Williams et al., 2000, ASB2012-12053). This review of subsequent glyphosate genotoxicity publications includes analysis of study methodology and incorporation of all the findings into a weight of evidence for genotoxicity. Two publications provided limited additional support for the conclusion that glyphosate and glyphosate based formulations (GBFs) are not active in the gene mutation assay category. The weight of evidence from *in vitro* and *in vivo* mammalian chromosome effects studies supports the earlier conclusion that glyphosate and GBFs are predominantly negative for this end point category. Exceptions are mostly for unusual test systems but there are also some unexplained discordant positive results in mammalian systems. Several reports of positive results for the SCE and comet DNA damage endpoints have been published for glyphosate and GBFs. The data suggest that these DNA damage effects are likely due to cytotoxic effects rather than DNA reactivity. This weight of evidence review concludes that there is no significant *in vivo* genotoxicity and mutagenicity potential of glyphosate or GBFs that would be expected under normal exposure scenarios.

B.6.4.8.2 General review and analysis considerations

The published studies for review consideration were identified by literature searches for published reports containing references to glyphosate or glyphosate based formulations (GBFs) that also contained searchable terms which indicated that genotoxicity studies were performed. Literature search utilised Chemical Abstracts (provided by Chemical Abstracts Service, a division of the American Chemical Society) and Web of Knowledge (Thompson Reuters), using the following modules: Web of ScienceSM, BIOSIS Previews®, MEDLINE®, and CAB Abstracts® (CABI) abstracting services. Search criteria were as follows (glyphosate acid and the various salts): glyphosat* OR glifosat* OR glyfosat* OR 1071-83-6 OR 38641-94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR 40465-66-5 OR 69254-40-6 OR (aminomethyl w phosphonic*) OR 1066-51-9. Each identified publication was evaluated to verify that it contained original results of one or more genotoxicity studies on glyphosate or GBFs. Emphasis was placed on publications in peer-reviewed journals and abstracts or other sources with incomplete information were not considered. Reviews without original data were not considered for evaluation; however, these reviews were examined to determine if there were any cited publications that had not been detected in the literature searches.

Each relevant publication was examined using several criteria to characterize the scientific quality of the reported genetic toxicology studies. Useful, objective criteria for this purpose were international guidelines for genetic toxicology studies developed by expert groups. These include principles for conducting studies, reporting results and analyzing and interpreting data. Some of the principles of the guidelines are generally applicable to categories of studies or all studies while others are specific for a particular type of test system and end point. Some of the specific types of studies encountered in the review do not yet have international guidelines; however, some of the guideline elements should be generically applicable to these studies. The guidelines for genetic toxicology tests developed for the Organisation for Economic Cooperation and Development (OECD) are a pre-eminent source

of internationally agreed and expert guidelines. Other regulatory international and national regulatory genetic toxicology testing guidance are usually concordant with the OECD guidelines. Table B.6.4-27 presents some key OECD guideline criteria that were found to be relevant to analysis of the studies considered in this review.

Comparison of the published studies to the criteria in guidelines used for regulatory purposes does not represent an absolute judgment standard but it does serve to provide one means of characterization of the various published studies. Some of the criteria are rarely met in scientific publications. For example, data for individual cultures and individual animals are not commonly included in publications in scientific journals. These data are presumably collected but are usually summarised as means with a measure of variance for the treatment and control groups. This is not considered to be a significant omission in a scientific publication. However, other guideline features are more essential in demonstrating scientific quality standards and should be considered as having greater weight in evaluating a study. For example, there are consistent recommendations that assays involving visual scoring (e.g. chromosome aberration, micronucleus and sister chromatid exchange) should use slides that are independently coded so that scoring is performed without knowledge of the treatment or control group being scored. This guidance is good scientific practice and studies that do not include a description of coding or “blind” scoring in the methodology would appear to have a deficiency either in the methodology or the description of the methodology used. Other examples of guideline features that have clear experimental scientific value are the use of concurrent negative and positive controls and concurrent measurement and reporting of toxicity endpoints in main experiments, especially in *in vitro* mammalian cell assays.

Test materials, as described in the publications, were reviewed by industry experts to identify any publicly available and useful information on composition for the reported formulations to assist in interpreting the relevance of findings to glyphosate and/or formulation components. It should be noted that a common problem encountered in the published literature is the use of the terms “glyphosate”, “glyphosate salt” or “Roundup” to indicate what may be any GBF that contains additional components such as surfactants. Published results from studies with different formulations have sometimes been incorrectly or inappropriately attributed to the active ingredient. The original Roundup formulation (MON 2139), containing 41 % isopropyl amine glyphosate salt and 15.4 % MON 0818 (a polyethoxylated [REDACTED] based surfactant blend), is no longer sold in many markets. However, other glyphosate based formulations are sold under the Roundup brand name with varying glyphosate forms, concentrations and surfactant systems. Clear identification of the test material is very important in toxicology studies because toxicity of formulations can be dramatically different than the active ingredient. The fact that test materials identified as Roundup formulations may actually have different compositions should be considered when comparing results of different studies. A major consideration, especially for DNA damage endpoints and for *in vitro* mammalian cell assays, is an assessment of whether observed effects might be due to toxicity or extreme culture conditions rather than indicating DNA-reactive mediated processes. Relevant considerations include control of medium pH and osmolality for *in vitro* mammalian cell studies and whether effects are observed only at cytotoxic doses or in association with severe toxicity to the test system. Other important generic considerations in evaluating experimental results of each published study are evidence of experimental reproducibility and whether a biologically plausible dose response has been demonstrated.

Table B.6.4-27: Genetic Toxicology Test Guideline Criteria






Area	Guidance	Reference
All studies	Test material purity and stability should be reported	OECD 471 (1997) OECD 473 (1997)
	Concurrent negative and positive controls should be included with each assay	
Assays with visual scoring	All slides should be independently coded before analysis (i.e. scored without knowledge of the treatment or control group)	OECD 473 (1997) OECD 479 (1986)
<i>In vitro</i> mammalian cell assays	Assay should be usually be conducted in the presence and absence of an appropriate exogenous metabolic activation system	OECD 473 (1997)
	Cytotoxicity should be determined in the main experiment	
	At least three analyzable concentrations should be used	
	Maximum dose determined by toxicity or 5 µg/ml, 5 mg/ml or 10 mM for soluble non-toxic test materials	
	Individual culture data should be provided	
<i>In vivo</i> mammalian assays	Five analyzable animals per group. Single sex may be used if there are no substantial difference in toxicity between sexes	OECD 475 (1997) OECD 474 (1997)
	Limit dose for non-toxic substances of 2000 mg/kg for treatments up to 14 days and 1000 mg/kg for treatments longer than 14 days	
<i>In vitro</i> chromosome aberration	Treatment for 3-6 hours in one experiment and harvest at 1.5 cell cycles. If negative a second experiment with continuous treatment for 1.5 cell cycles	OECD 473 (1997)
	Scoring of at least 200 metaphases ideally divided between duplicate cultures	
<i>In vitro</i> sister chromatid exchange	Treatment for 1-2 hours up to two cell cycles with harvest after two cell cycles in the presence of bromodeoxyuridine	OECD 479 (1986)
	Scoring of 25 metaphases per culture (50 per treatment group)	
<i>In vitro</i> micronucleus	Most active agents detected by treatment for 3-6 hours with harvest at 1.5-2 cell cycles after treatment. An extended treatment for 1.5-2 cycles in the absence of metabolic activation is also used	OECD 487 (2010)
	Scoring of at least 2000 binucleated cells or cells for micronuclei for each treatment or control group	
<i>In vivo</i> bone marrow chromosome aberration	Single treatment with first harvest at 1.5 cell cycles after treatment and second harvest 24 hour later or single harvest 1.5 cycles after last treatment for multiple daily treatments	OECD 475 (1997)
	Three dose levels usually recommended except when limit dose produces no toxicity	
	Concurrent measures of animal toxicity and toxicity to target cells	
	At least 100 cells analyzed per animal	
	Individual animal data should be reported	
<i>In vivo</i> erythrocyte micronucleus	Three dose levels for first sampling time	OECD 474 (1997)
	Treatment once with at least 2 harvests usually at 24 and 48 h after treatment or one harvest 18-24 h after final treatment if two or more daily treatments are used	
	Scoring of 2000 immature erythrocytes per animal or 2000 mature erythrocytes for treatments of 4 weeks or longer	

Table B.6.4-28 presents a summary of genotoxicity test results for glyphosate and GBFs published subsequent to (2000, ASB2012-12053). Test results are organised by the major genotoxicity assay categories of gene mutation, chromosome effects and DNA damage and other end points. Major features presented for each publication are the assay

endpoint, the test system, the test material, the maximum dose tested and comments relevant to the reported conduct and results of the assay. For brevity, earlier reviewed individual publications of genotoxicity study results are referred to by citation of (██████████ 2000, ASB2012-12053) rather than the original references reviewed in (██████████ 2000, ASB2012-12053).

Table B.6.4-28: Genetic toxicology studies of glyphosate and glyphosate formulations published on or after 2000

End point	Test System	Test Material	Maximum Dose	Result	Comment	Reference
<i>In Vitro Gene Mutation</i>						
Point mutation	Ames strains	Perzocyd 10 SL formulation	2 µg/plate (toxic)	Negative	TA1535 not used	Chruscielska et al., 2000, (ASB2013-9830)
Wing spot test	Drosophila	glyphosate (96%)	10 mM in larval stage	Negative, inconclusive ^c	Negative or inconclusive in crosses not sensitive to recombination events	Kaya et al., 2000, (ASB2013-9832)
<i>In Vitro Chromosome Effects—Mammalian Systems</i>						
Cytokinesis block micronucleus	Bovine lymphocytes	Glyphosate formulation (62% glyphosate Monsanto source)	560 µM 48 h –S9	Positive?	PH, MA, SC, TO	Piesova, 2004 (ASB2012-12001)
Cytokinesis block micronucleus	Bovine lymphocytes	Glyphosate formulation (62% glyphosate Monsanto source)	560 µM 48 h –S9 2 h –S9 2 h +S9	Positive? Negative Negative	PH, SC, TO	Piesova, 2005 (ASB2012-12000)
Chromosome aberration	Mouse spleen cells	herbazed formulation	50 µM?	Positive	Concentrations used not clear. PH, MA, SC, TO, RE	Amer et al., 2006 (ASB2012-11539)
Chromosome aberration	Bovine lymphocytes	Glyphosate formulation (62% glyphosate) Monsanto source	1.12 mM (toxic) (24 h)	Negative	Chromosome 1 FISH analysis. PH, MA, PC, SC, TO, RE	Holeckova, 2006 (ASB2012-11847)
Chromosome aberration	Bovine lymphocytes	Glyphosate formulation (62% glyphosate) Monsanto source	1.12 mM (toxic) (24 h)	Negative	PH, MA, SC, RE	Sivikova and Dianovsky, 2006 (ASB2012-12029)
Chromosome aberration	Human lymphocytes	Glyphosate (96%)	6 mM (not toxic)	Negative	MA, IC, RE	Manas et al., (2009 ASB2012-11892)

End point	Test System	Test Material	Maximum Dose	Result	Comment ^a	Reference
Cytokinesis block micronucleus	Human lymphocytes	Glyphosate (technical, 96%)	580 µg/mL (toxic) (est. 3.43 mM)	Negative (-S9) Positive (+S9)	SC, RE	Mladinic et al., 2009 (ASB2012-11906)
Cytokinesis block micronucleus	Human lymphocytes	Glyphosate (technical, 96%)	580 µg/mL (toxic) (est. 3.43 mM)	Negative (-S9) Positive (+S9)	SC, RE	Mladinic et al., 2009 (SB2012-11907)
<i>In Vitro Chromosome Effects—Non Mammalian Systems</i>						
Chromosome aberration	Onion root tip meristem	Roundup formulation (Bulgaria)	1% active ingredient (estimated 4.4-5.9 mM)	Negative	TO, IC, RE	Dimitrov et al., 2006 (SB2012-11607)
Micronucleus	Onion root tip meristem	Roundup formulation (Bulgaria)	1% active ingredient (estimated 4.4-5.9 mM)	Negative	TO, RE	Dimitrov et al., 2006 (SB2012-11607)
<i>In Vivo Chromosome Effects—Mammalian Systems</i>						
Bone marrow erythrocyte micronucleus	Mouse	Glyphosate	300 mg/kg i.p. Perzocyd 10 SL formulation	Negative Negative	DL, TO, SC, IM, RE DL, TO, SC, IM, RE	 2000, (ASB2013-9830)
Bone marrow erythrocyte micronucleus	Mouse	Roundup 69 formulation	2 x 200 mg/kg i.p.	Negative	TO, SC, IE, RE	 2000 (ASB2013-11477)
Bone marrow erythrocyte micronucleus	Mouse	Roundup™ formulation (Monsanto)	2 x 200 mg/kg i.p.	Negative	TO, SC, IE, RE	 2002 (SB2012-11834)
Bone marrow Chromosome aberration	Rabbit	Roundup™ formulation	750 ppm in drinking water	Positive?	DL, PC, TO, SC, IC	 2005 (ASB2012-11841)
Bone marrow Chromosome aberration	Mouse	Herbazed formulation (84% glyphosate)	50 mg/kg i.p. (1,3, 5 days) 100 mg/kg oral (1,7, 14, and 21 days)	Negative Positive	TO, SC, RE	 2006 (ASB2012-11539)

End point	Test System	Test Material	Maximum Dose	Result	Comment ^a	Reference
Spermatocyte Chromosome aberration	Mouse	Herbazed formulation (84% glyphosate)	50 mg/kg i.p. (1,3, 5 days) 100 mg/kg oral (1,7, 14, and 21 days)	Negative Positive	TO, SC, RE	[REDACTED] 2006 (ASB2012-11539)
Bone marrow Chromosome aberration	Mouse	Roundup formulation (Bulgaria)	1080 mg/kg p.o. (1/2 LD50)	Negative	DL, TO, IC, RE	[REDACTED] (ASB2012-11607)
Bone marrow erythrocyte micronucleus	Mouse	Analytical glyphosate (96%)	2 x 200 mg/kg i.p.	Positive	Erythrocytes scored? TO, SC, IC, RE	[REDACTED] 2009 (ASB2012-11892)
Bone marrow Chromosome aberration	Mouse	Roundup TM formulation (Monsanto)	50 mg/kg i.p.	Positive	DL, SC, IC, RE	[REDACTED] 2009 (ASB2012-12005)
<i>In Vivo Chromosome Effects—Non-Mammalian Systems</i>						
Erythrocyte micronucleus	Oreochromis niloticus (Tilapia)	Roundup 69	170 mg/kg i.p. (maximum tolerated)	Negative? ^c	TO, RE	[REDACTED], 2000 (ASB2013-11477)
Wing spot test	Drosophila	Glyphosate (96%)	10 mM in larval stage	Positive/inconclusive ^b		[REDACTED] 2000 (ASB2013-9832)
Erythrocyte micronucleus	Tilapia	Roundup TM formulation (Monsanto)	170 mg/kg (abdominal injection)	Positive	TO, RE	[REDACTED], 2002 (ASB2012-11834)
Erythrocyte micronucleus	Crassus auratus (goldfish)	Roundup formulation	15 ppm glyphosate in water (2, 4 and 6 days)	Positive	TO, IE, RE	[REDACTED] 2007 (ASB2012-11587)
	Prochilodus lineatus (tropical fish)	Roundup TM formulation (75% of 96 h LC50)	10 mg/l (6, 12 and 24 h) in water	Negative	DL, TO, SC, RE	[REDACTED] 2008 (ASB2012-11586)
Erythrocyte micronucleus	Caiman eggs	Roundup [®] Full II formulation	1750 ug/egg	Positive	RE	[REDACTED] 2009 (ASB2012-12002)
Erythrocyte micronucleus	Caiman eggs	Roundup [®] Full II formulation	Sprayed 2x with 100 litres of 3%/ha 30 days apart	Positive	DL, TO, RE	[REDACTED] 2009 (ASB2012-12002)

End point	Test System	Test Material	Maximum Dose	Result	Comment ^a	Reference
<i>In Vitro DNA Damage Mammalian Systems</i>						
Alkaline SCGE	GM38 human fibroblasts and HT1090 human fibrosarcoma	Glyphosate (technical grade)	6.5 mM	Positive	MA, PH, TO, SC, RE	Monroy et al., 2005 (ASB2012-11910)
Sister chromatid exchange	mouse spleen cells	herbazed formulation	50 µM?	Positive	Concentrations used not clear MA, PH, TO, SC, RE	Amer et al., 2006 (ASB2012-11539)
Sister chromatid exchange	bovine lymphocytes	Glyphosate formulation (62% glyphosate, Monsanto)	1.12 mM (toxic)	Positive	PH, SC, RE	Sivikova and Dianovsky, 2006 (ASB2012-12029)
Alkaline single cell gel electrophoresis (SCGE, comet)	Hep-2 cells	Glyphosate (analytical, 96%)	7.5 mM (limited by toxicity)	Positive	MA, PH, RE	Manas et al., 2009 (ASB2012-11892)
Alkaline SCGE	Human lymphocytes	Glyphosate (technical, 96%)	580 µg/ml (toxic) (est. 3.43 mM)	Positive (-S9) Positive (+S9)		Mladinic et al., 2009 (ASB2012-11906)
<i>In Vitro DNA Damage Non-Mammalian Systems</i>						
SOS	E. coli	Roundup BIO formulation	2.5 µg/sample	Positive		Raipulis et al. 2009 (ASB2012-12008)
Alkaline SCGE	Tradescantia flowers and nuclei	Glyphosate (technical, 96%)	700 µM	Positive	PH, SC	Alvarez-Moya et al., 2011 (ASB2012-11538)
Bone marrow SCE	Mouse	herbazed formulation (84% glyphosate)	200 mg/kg p.o.	Positive	TO, SC, RE	Amer et al., 2006 (ASB2012-11539)
Sperm abnormality	Mouse	herbazed formulation (84% glyphosate)	200 mg/kg p.o. (5 days)	Positive	TO, SC, RE	Amer et al., 2006 (ASB2012-11539)
<i>In Vivo DNA Damage Non-Mammalian Systems</i>						
Erythrocyte alkaline SCGE	Crassus auratus (goldfish)	Roundup formulation	15 ppm glyphosate in water (2, 4 and 6 days)	Positive	TO, RE	[REDACTED], 2007 (ASB2012-11587)
Erythrocyte and gill cell alkaline SCGE	Prochilodus lineatus (tropical fish)	Roundup TM formulation (75% of 96 h LC ₅₀)	10 mg/l (6, 12 and 24 h) in water	Positive	DL, TO, RE	[REDACTED], 2008 (ASB2012-11586)
Erythrocyte alkaline SCGE	Caiman eggs/hatchlings	Roundup [®] Full II	1750 µg/egg	Positive	RE	[REDACTED], 2009 (ASB2012-11585)

End point	Test System	Test Material	Maximum Dose	Result	Comment ^a	Reference
		formulation				12002)
Erythrocyte alkaline SCGE	European eel	Roundup formulation	166 µg/liter	Positive	DL, SC, RE	2010 (ASB2012-11836)
Erythrocyte alkaline SCGE	Caiman eggs/hatchlings	Roundup® Full II formulation	Sprayed 2x with 100 l of 3%/ha 30 days apart	Positive	DL, RE	2009 (ASB2012-12002)

- ^a MA, Mammalian metabolic activation system not used and short exposure not used;
 PH, no indication of pH or osmolality control;
 DL, less than three dose levels used; PC, no concurrent positive control;
 TO, no concurrent measurement of toxicity reported or toxicity not observed for highest dose level;
 SC, independent coding of slides for scoring not indicated for visually scored slides;
 IC, less than 200 cells scored per treatment or less than 100 metaphases scored per animal for chromosome aberrations.;
 IE, less than 2000 erythrocytes scored per animal;
 RE, results not reported separately for replicate cultures or individual animals;
^b Positive for small wing spots only in one cross. Negative or inconclusive for all spot categories for three other crosses.
^c Statistically significant increase in micronucleated PCE frequency only at mid dose level but overall result judged negative.

B.6.4.8.3 Structure Activity Analysis

Glyphosate was evaluated using Derek for Windows (Lhasa Ltd., Leeds, UK, Version 11.0.0, October 24, 2009). No structural alerts were identified for chromosome damage, genotoxicity, mutagenicity or carcinogenicity. This small molecule consists of the amino acid, glycine, joined with a phosphonomethyl group. These moieties are not known to be genotoxic; therefore, the lack of structure activity alerts for glyphosate is expected.

B.6.4.8.4 Gene Mutation

As reviewed by Williams et al., (2000, ASB2012-12053), most gene mutation studies for glyphosate and GBFs were negative. Gene mutation assays included numerous Ames/*Salmonella* and *E. coli* WP2 bacterial reversion assays, *Drosophila* sex-linked recessive lethal assays and a CHO/HGPRT *in vitro* mammalian cell assay. Of fifteen gene mutation assays reported, there were only two positive observations. A reported positive Ames/*Salmonella* result for Roundup formulation was not replicated in numerous other studies. There was one report of a positive result for a GBF in the *Drosophila* sex-linked recessive lethal assay but this was contradicted by a negative result for the same GBF in this assay reported by another laboratory. Further, the positive study had some features that hampered interpretation, including the lack of concurrent negative controls (Williams et al., 2000). Subsequent to the Williams et al. (2000, ASB2012-12053) review only two gene mutation studies have been reported (Table B.6.4-28). One negative Ames/*Salmonella* assay result was reported for a GBF of undefined composition, Percozyd 10 SL (Chruscielska et al., 2000, ASB2013-9820). Although this result is consistent with a large number of negative Ames/*Salmonella* results for glyphosate and GBFs, the reported study results have significant

limitations. One of the recommended test strains, TA1535, was not used and results were only presented as “-“ without presentation of revertant/plate data. A positive result for glyphosate was reported in the *Drosophila* wing spot assay which can indicate both gene mutation and mitotic recombination endpoints (Kaya et al., 2000, ASB2013-9832). Small increases in small wing spot frequencies were observed in one of four crosses of larvae treated with up to 10 mM glyphosate. The lack of a positive response in the balancer-heterozygous cross offspring, which are insensitive to mitotic recombination events, suggests that there is no evidence for effects on gene mutation endpoint events such as intragenic mutations or deletions in this publication.

These gene-mutation publications add very limited data to the weight of evidence conclusion that glyphosate and GBFs do not pose significant risk for gene mutation.

B.6.4.8.5 Chromosome effects

Assays to detect chromosome effects such as structural chromosome aberrations and micronucleus incidence constitute a second major genotoxicity end point category. A large number of publications with chromosome effects endpoints have been reported since the [REDACTED] (2000, ASB2012-12053) review. These are described in Table B.6.4-28 and are separated into various test system categories which include *in vitro* cultured mammalian cell assays, *in vitro* tests in non-mammalian systems, *in vivo* mammalian assays and *in vivo* assays in non-mammalian systems. A *Drosophila* wing spot test (discussed previously) is also included in this category because results are relevant to somatic recombination.

B.6.4.8.5.1 *In vitro* chromosome effects

Two human and one bovine *in vitro* peripheral lymphocyte chromosome aberration studies of glyphosate were considered in the earlier review (Williams et al., 2000, ASB2012-12053). One human lymphocyte *in vitro* study had negative results for glyphosate tested up to approximately 2-3 mM (calculated from reported mg/ml) in the absence and presence of an exogenous mammalian activation system. The other two studies with human and bovine lymphocytes and no metabolic activation system reported positive results at concentrations more than two orders of magnitude lower. The earlier review noted several other unusual features about the positive result studies including an unusual exposure protocol and discordant positive results for another chemical found negative in other laboratories.

As indicated in Table B.6.4-28 both positive and negative results have been reported for glyphosate and GBFs in the nine *in vitro* chromosome effects assays published after the Williams et al. (2000, ASB2012-12053) review. It is noteworthy that many of these studies have various deficiencies in conduct or reporting compared to internationally accepted guidelines for conduct of *in vitro* chromosome aberration or micronucleus studies (see Table B.6.4-27). Perhaps the most significant deficiency was that coding and scoring of slides without knowledge of the treatment or control group was not indicated in seven of nine publications. This could be a deficiency in conducting the studies or perhaps a deficiency in describing methodology in the publications. Other common deficiencies included failure to indicate control of exposure medium pH, no use of exogenous metabolic activation and no reporting of concurrent measures of toxicity.

Results for glyphosate active ingredient

Three publications reported testing of technical glyphosate for micronucleus or chromosome aberration endpoints in cultured human lymphocytes (Manas et al., 2009, ASB2012-11892;

Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012-11907). Negative results for the micronucleus or chromosome aberration end points were observed in the absence of exogenous metabolic activation (S9) in all three publications. The maximum exposure concentration in the absence of S9 was in the range of 3-6 mM in these studies. Two publications by one author reported cytokinesis block micronucleus results for cultured bovine lymphocytes treated with what was reported as 62 % by weight isopropyl amine salt of glyphosate from a Monsanto Belgium source (Piesova, 2004, ASB2012-12001; Piesova, 2005, ASB2012-12000). This test material appears to be a manufacturing batch of the isopropylamine salt of glyphosate in water without surfactants, which is not sold as a GBF. In one publication no statistically significant increases in binucleated cell micronucleus frequency were observed with 24 hours of treatment (Piesova, 2004, ASB2012-12001). For 48 hours of treatment a statistically significant increase in micronucleus frequency was observed in one donor at 280 μ M but not at 560 μ M and in a second donor at 560 μ M but not 280 μ M. The second publication reported negative results for the cytokinesis block micronucleus assay in bovine lymphocytes incubated with glyphosate formulation up to 560 μ M for two hours in the absence and presence of a mammalian metabolic activation system (Piesova, 2005, ASB2012-12000). This publication also reported positive results for 48 hours of treatment without S9. Curiously, in this second publication the same inconsistent dose response pattern was observed in which a statistically significant increase in micronucleus frequency was observed in one donor at 280 μ M but not at 560 μ M and in a second donor at 560 μ M but not 280 μ M. The lack of a consistent dose response pattern between donors suggests that the results with 48 hours of treatment are questionably positive. Two other publications found negative results for the chromosome aberration endpoint in cultured bovine lymphocytes treated with what appears to be the same test material of 62 % by weight isopropylamine salt of glyphosate from a Monsanto Belgium source, (Holeckova, 2006, ASB2012-11847; Sivikova and Dianovsky, 2006, ASB2012-12029). Both the studies used a maximum concentration of 1.12 mM which was reported to cause a decrease in mitotic inhibition of >50 %. These two studies have several limitations including that an exogenous mammalian metabolic activation system was not used for chromosome aberration and scoring was not reported to be on coded slides. In addition, Holeckova (2006, ASB2012-11847) only examined effects detectable by staining of chromosome 1 and did not report positive control results (Holeckova, 2006, ASB2012-11847). Despite these limitations and the variable donor results, the results from these two studies are generally consistent with a lack of chromosome aberration effects of the isopropylamine salt of glyphosate on *in vitro* cultured mammalian cells in several experiments using high, toxic dose levels and exposures of 2-24 hours in the absence of S9.

One laboratory reported increases in cytokinesis-blocked micronucleus frequency in cultured human lymphocytes exposed to glyphosate for 4 hours in the presence of an exogenous human liver metabolic activation system (S9) in two publications (Mladinic et al., 2009a; Mladinic et al., 2009b). In both publications a statistically significant increase in micronuclei was observed with S9 at the highest dose level of glyphosate tested (580 μ g/mL, \approx 3.4 mM). Increased proportions of centromere- and DAPI-positive micronuclei were observed for the high dose with S9 suggesting that the induced micronuclei were derived from chromosomes rather than chromosome fragments. Statistically significant increases in the frequency of nuclear abnormalities (buds and bridges) and DNA strand breakage were also observed at the highest dose tested in both publications. In parallel experiments cytotoxic effects such as early apoptosis, late apoptosis and necrosis were observed and these effects were uniquely or preferentially observed in the presence of S9 and at the highest dose level tested (Mladinic et al., 2009, ASB2012-11906). Also, the negative control level of such end points as necrosis and alkaline SCGE tail moment was significantly increased in the presence of S9 (Mladinic et

al., 2009, ASB2012-11906). It should be noted that glyphosate is mostly excreted unmetabolised *in vivo* in mammals with only very small levels of aminomethylphosphonic acid (AMPA) or an AMPA-related structure observed (Mladinic et al., 2009, ASB2012-11542; Brewster et al., 1991, TOX9551791). These observations suggest that the observations of S9 mediated effects by Mladinic et al. are not likely to be due to *in vivo* relevant metabolites. The preponderance of *in vitro* genotoxicity studies conducted with exogenous mammalian metabolic activation systems has been negative, including a previously reviewed chromosome aberration study in human lymphocytes conducted up to a similar dose level (Williams et al., 2000, ASB2012-12053) and a bovine lymphocyte cytokinesis block micronucleus study (Piesova, 2005, ASB2012-12000). Overall these results suggest the possibility of a weak aneugenic rather than clastogenic (chromosome breaking) effect occurring in the presence of S9 at high dose levels of glyphosate. The pattern of activity as well as the failure to observe activity in several other *in vitro* genotoxicity assays conducted with S9 suggests that the activity observed in the Mladinic et al. studies does not have a significant weight of evidence for *in vitro* genotoxicity and is not likely to be relevant to *in vivo* genotoxicity.

The recently published results for mammalian *in vitro* chromosome aberration and micronucleus assays demonstrate a weight of evidence that technical glyphosate and glyphosate salt concentrates are negative for these end points in cultured mammalian cells in the absence of an exogenous mammalian metabolic activation system. Five publications from four laboratories report negative *in vitro* mammalian cell chromosome or micronucleus results in the absence of exogenous activation while three publications from two laboratories report positive results. These results reinforce the Williams et al. (2000, ASB2012-12053) conclusion that positive chromosome aberration results reported for glyphosate in cultured human lymphocytes in the absence of an exogenous metabolic activation system are aberrant. Recent reports of positive chromosome aberration and micronucleus results for glyphosate in the presence of an exogenous mammalian activation system in cultured human lymphocytes in one laboratory (Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012-11907) have no substantial reproducibility verification from other laboratories in the recent *in vitro* chromosome effects studies considered in this review because most of the studies performed by other laboratories (Table B.6.4-28) did not employ an exogenous mammalian activation system. These results are discordant with one previously reviewed result demonstrating a negative result for glyphosate in cultured human lymphocytes with mammalian metabolic activation using the chromosome aberration endpoint (Williams et al 2000, ASB2012-12053) and a negative result in the presence of S9 for the micronucleus endpoint in bovine lymphocytes (Piesova, 2005, ASB2012-12000). The numerous consistent negative results for glyphosate and GBFs in gene mutation studies which employed exogenous mammalian metabolic activation and careful examination of the data suggests that the positive results indicate a possible threshold aneugenic effect associated with cytotoxicity rather than a DNA-reactive mechanism resulting in chromosome breakage. Thus, the weight evidence for the *in vitro* chromosome effect assays indicates a lack of DNA-reactive clastogenic chromosome effects.

Results for GBFs

Amer et al. (2006, ASB2012-11539) reported positive *in vitro* chromosome aberration effects in mouse spleen cells for a formulation described as herbazed, which was reported to contain 84 % glyphosate and 16 % solvent, an unusually high glyphosate concentration for a formulation. The test material is not further characterised, lacking description of the glyphosate salt form and inert ingredients. The glyphosate concentrations used in the study are not clear because there are different descriptions of the concentration units (M or M glyphosate/ml medium) in the publication. Thus, the maximum concentration might have

been 5×10^{-5} M (50 μ M) or 5×10^{-5} M glyphosate/mL medium (50 mM). The former concentration, which was reported as toxic, would indicate effects at concentrations well below those typically found toxic for GBFs in cultured mammalian cells. The latter level of 50 mM would be well in excess of the limit level of 10 mM recommended in OECD guidelines (OECD473, 1997). In addition to a question about the concentration used there are several other limitations to the reported study including no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Given these limitations, the uncertainty about the concentrations used and the nature of the test material, these results should not be considered to have significant relevance or reliability with respect to glyphosate or GBFs.

In addition to *in vitro* mammalian cell studies there is also a report of negative results for the chromosome aberration and micronucleus endpoints in onion root tips incubated with a Roundup formulation (Dimitrov et al., 2006, ASB2012-11607). The maximum exposure concentration (stated as 1 % active ingredient) is estimated to be on the order of 4-6 mM. This study did not employ an exogenous mammalian metabolic activation system; however, it does provide evidence for a lack of chromosome effects for glyphosate and a GBF in a non-mammalian *in vitro* system. The result agrees with earlier reported negative onion root tip chromosome aberration results for glyphosate but is discordant with earlier reported positive results for a Roundup GBF in this system (Williams et al., 2000, ASB2012-12053).

B.6.4.8.5.2 *In vivo* Chromosome Effects—Mammalian Systems

The [REDACTED] (2000, ASB2012-12053) glyphosate toxicity review presented results from *in vivo* mammalian chromosome effect assays. Results from several mouse bone marrow erythrocyte micronucleus studies of glyphosate and GBFs (e.g. Roundup, Rodeo and Direct) were negative for micronucleus induction. These included studies from different laboratories mostly following modern guidelines. The intraperitoneal (i.p.) route was used for most of the negative studies and maximum doses for many of the studies were toxic or appropriately close to LD50 values. In addition to i.p. studies a 13 week mouse feeding study was also negative for the micronucleus endpoint with an estimated maximum daily glyphosate dose of over 11,000 mg/kg/day. There was one published report of a weak positive mouse bone marrow micronucleus response observed for glyphosate and Roundup GBF. This study, which employed a smaller number of animals per group than other negative studies, was clearly aberrant from the numerous other negative studies not only in micronucleated cell frequency finding but also the finding of altered polychromatic erythrocyte to normochromatic erythrocyte (PCE/NCE) ratios. The overall weight of evidence from the earlier reviewed studies was that glyphosate and GBFs were negative in the mouse bone marrow erythrocyte micronucleus assay. The earlier review also noted a negative mouse dominant lethal result for glyphosate administered by gavage at a maximum dose level of 2000 mg/kg.

As indicated in Table B.6.4-28, there are numerous subsequent publications of *in vivo* mammalian chromosome effects assays. With one exception, all of the *in vivo* mammalian studies were conducted in the mouse using either the bone marrow chromosome aberration or micronucleus endpoints. It should be noted that there are some fairly consistent limitations in the reported conduct of these studies compared to OECD guidelines. In most studies concurrent indications of toxicity (other than effects on the bone marrow) are not reported, coding of slides for scoring is not reported, individual animal data are not reported and fewer than recommended cells or metaphases per animal were scored. Other limitations encountered include use of only a single or two dose levels rather than three dose levels.

Results for glyphosate active ingredient

Two publications reported results for glyphosate in the mouse bone marrow erythrocyte micronucleus assay. Negative results were reported in one study which used a dose of 300 mg/kg of glyphosate administered once i.p. with sacrifices at 24, 48 and 74 hours after dosing (██████████ 2000, ASB2013-9820). This study had some limitations including the use of only one dose level, no reporting of toxicity other than PCE/NCE ratio, no reported coding of slides for scoring and scoring of 1000 PCE's per animal (scoring of 2000 PCE's per animal is recommended by OECD guidelines). A second publication reported positive results for glyphosate administered at 50, 100 and 200 mg/kg via i.p. injections repeated at 24 hours apart with sacrifice 24 hours after the second dose (██████████ 2009, ASB2012-11892). A statistically significant increase in micronucleated erythrocytes was observed in the high dose group. This study had limitations comparable to the negative study. A more significant potential difficulty with this second publication is that "erythrocytes" rather than polychromatic erythrocytes were indicated as scored for micronuclei. This does not appear to be a case of using "erythrocytes" to mean polychromatic erythrocytes because the term "polychromatic erythrocytes" is used elsewhere in the publication describing measurements of PCE/NCE ratios. Scoring of total erythrocytes instead of immature polychromatic erythrocytes for micronuclei would be inappropriate in an assay with the stated treatment and harvest times because of the transient nature of micronucleated PCE's in bone marrow (OECD474, 1997).

There is no definitive explanation for the discrepancy between the two publications. Although one study used a single dose with multiple harvest times and the second used two doses and a single harvest time, both are acceptable protocols and would not be expected to lead to such discordant results (OECD474, 1997). The negative result reported for the 13 week feeding study in the earlier review (██████████ 2000, ASB2012-12053) confirms that positive results are not simply due to repeat dosing. The reported negative result (██████████ 2000, ASB2013-9820) seems to be in accord with a majority of earlier reviewed mouse bone marrow micronucleus studies of glyphosate using similar doses and the i.p. or feeding routes (██████████ 2000, ASB2012-12053). Also, the apparent scoring of micronuclei in erythrocytes rather than just polychromatic erythrocytes raises a significant methodological question for the reported positive study.

Results for GBFs

There are several publications reporting *in vivo* mammalian bone marrow chromosome aberration and micronucleus endpoint results for Roundup GBFs. Three publications report negative results for Roundup branded GBF in mouse chromosome aberration or micronucleus assays. Negative results were reported for two different Roundup branded GBFs administered at 2 x 200 mg/kg i.p. in mouse bone marrow erythrocyte micronucleus assays (██████████ 2000, ASB2013-11477; ██████████ 2002, ASB2012-11834). The second study did not report coding of slides for scoring. Another publication reported negative results in mouse bone marrow studies for both the chromosome aberration and erythrocyte micronucleus endpoints (██████████ 2006, ASB2012-11607) using a dose of 1080 mg/kg administered orally (p.o.). In contrast, one publication reported positive results for Roundup GBF in mouse bone marrow for the chromosome aberration and erythrocyte micronucleus endpoints using a single maximum dose of 50 mg/kg i.p. (██████████ 2009, ASB2012-12005). Both the positive results and the magnitude of the increases in the chromosome aberration and micronucleus endpoint reported in this study are remarkably discordant with other reported results for Roundup and other GBFs in mouse bone marrow chromosome aberration and erythrocyte studies in a number of laboratories and publications

(Table B.6.4-28 and [REDACTED] 2000, ASB2012-12053). The reasons for this discordance are not clear. One unusual feature of the positive study is that the Roundup GBF was administered in dimethylsulfoxide. This is an unusual vehicle to use in *in vivo* genotoxicity studies, particularly for glyphosate which is water soluble and especially so in a formulated product. A published toxicity study found that use of a dimethylsulfoxide/olive oil vehicle by the i.p. route produced dramatically enhanced toxicity of glyphosate formulation or the formulation without glyphosate compared to saline vehicle and that the enhanced toxicity observed with this vehicle was not observed when the oral route was used ([REDACTED] 2008, ASB2012-11845). These observations suggest that use of DMSO as a vehicle for administration of formulation components by the i.p. route might produce unusual toxic effects that are not relevant to normally encountered exposures. Regardless of the reasons for the discordant positive results it is clear that a large preponderance of evidence indicates that GBFs are typically negative in mouse bone marrow chromosome aberration and erythrocyte assays.

One publication reported positive results for bone marrow chromosome aberration in rabbits administered Roundup GBF in drinking water at 750 ppm for 60 days ([REDACTED] 2005, ASB2012-11841). This study is relatively unique in terms of species and route of administration. The results do not report water intake in the test and control groups. Given the potential for water palatability issues with a formulated product, this is a significant shortcoming, as any effects noted may be attributable to dehydration. This study had further limitations including the use of only a single dose level and not coding slides for scoring. Examination of the chromosome aberration scoring results showed that large increases for the treated group were observed for gaps and “centromeric attenuation” which were included in the summation and evaluation of structural chromosome aberration effects. Ordinarily gaps are scored but are not recommended for inclusion in total aberration frequency and centromeric attenuation is not included in ordinary structural aberrations (OECD475, 1997). These unusual scoring and interpretive features raise significant questions about using this study to make conclusions about clastogenicity of the GBF tested.

Two other publications report *in vivo* mammalian chromosome aberration or micronucleus results for GBFs. An uncharacterized GBF, Percozyd 10L, was reported to be negative in a mouse bone marrow erythrocyte micronucleus assay ([REDACTED] 2000, ASB2013-8929 and ASB2013-8931). The maximum dose level tested, 90 mg/kg i.p., was reported to be 70 of the i.p. LD₅₀ as determined experimentally by the authors. This study had several limitations including use of less than three dose levels and no reported coding of slides for scoring. Positive results were reported for another uncharacterized GBF, herbazed, in mouse bone marrow and spermatocyte chromosome aberration studies ([REDACTED] 2006, ASB2012-11539). No statistically significant increases in aberrant cells were observed in bone marrow cells for i.p. treatment of 50 mg/kg for 1, 3 or 5 days or in spermatocytes for 1 or 3 days treatment. Statistically significant increases in frequency of spermatocytes with aberrations were reported for 5 days of treatment with 50 mg/kg (i.p.). Oral treatment of 50 mg/kg and 100 mg/kg were reported to produce increases in aberrant cell frequency in bone marrow cells after extended treatments (14 and 21 days) but not after shorter 1 and 7 day treatments. Similarly, significant increases in aberrant cell frequencies of spermatocytes were reported at 14 and 21 days of 50 mg/kg oral treatment (negative for 1 and 7 days treatment) and at 7, 14 and 21 days of 100 mg/kg treatment (negative for 1 day treatment). Although not a genotoxic endpoint per se, it should be noted that statistically significant increases in frequency of sperm with abnormal morphology were also observed in mice treated with 100 and 200 mg/kg p.o. for 5 days. The positive results for the uncharacterized herbazed GBF were only observed after extended oral treatments (bone marrow and spermatocytes) and extended i.p. treatments (spermatocytes). The fact that positive results were not observed in an erythrocyte

m micronucleus test of mice treated with glyphosate up to 50,000 ppm in feed for 13 weeks ([REDACTED] 2000, ASB2012-12053) provides direct evidence that extended glyphosate treatment by the oral route does not induce detectable chromosome effects. This treatment was longer and up to much higher glyphosate exposures than those used for the [REDACTED] (2006, ASB2012-11539) studies. Thus, it appears likely that these effects were due to some component(s) of the specific herbized GBF tested rather than glyphosate.

In vivo mammalian assays for chromosome effects are an important category for characterising genotoxicity that complements the gene mutation category. While some positive results have been reported the preponderance of evidence and published results are negative for glyphosate and GBFs.

B.6.4.8.5.3 *In vivo* Chromosome Effects—Non-Mammalian Systems

The [REDACTED] (2000, ASB2012-12053) review reported a few *in vivo* plant assays for chromosome effects in non-mammalian systems. These included negative results for glyphosate and positive results for Roundup GBFs for chromosome aberrations in an onion root tip assay and negative results for glyphosate with the micronucleus end point in a *Vicia faba* root tip assay.

Subsequent to the earlier review a number of publications reported results for erythrocyte micronucleus assays conducted on GBFs in several non-mammalian fish and reptile species with discordant results. One publication reported apparently negative results for the erythrocyte micronucleus test in *Oreochromis niloticus* (Nile tilapia) administered a test material described as Roundup 69 GBF, at an upper dose of 170 mg/kg i.p. ([REDACTED] 2000, ASB2013-11477). Although there was an increase in micronucleated erythrocyte frequency at the mid-dose level this was not observed at the high dose level and considerable variability in frequencies in different groups was noted. Negative results were also reported in another fish species (*Prochilodus lineatus*) exposed to 10 mg/liter Roundup branded GBF for 6, 24 and 96 hours ([REDACTED] 2008, ASB2012-11586). This concentration was reported to be 96 % of a 96 hour LC₅₀. Positive results were reported for the erythrocyte micronucleus assay conducted in the fish *Tilapia rendalii* exposed to 170 mg/kg i.p. of another Roundup GBF ([REDACTED] 2002, ASB2012-11834). Examination of the micronucleus frequencies in this publication indicated that the negative control micronucleus frequency was considerably lower than the frequencies for all but one of 21 treatment groups for 7 different test materials. This suggests an unusually low control frequency and at least one treatment group was statistically significantly elevated for each of the 7 test materials, including many instances where the statistically significant increases were not consistent with a biologically plausible dose response. The possibility that the apparently significant increases were due to a low negative control value should be considered for this publication. Another publication reported positive erythrocyte micronucleus results in goldfish (*Carassius auratus*) exposed to 5 to 15 ppm of a Roundup GBF for 2 to 6 days ([REDACTED] 2007, ASB2012-11587). The reasons for the discordant results are not clear for these fish erythrocyte micronucleus assays of Roundup GBFs. Although different species and GBF's were used in the different studies there were pairs of studies with positive and negative results that used similar treatment conditions (170 mg/kg i.p. or 10-15 mg/litre in water).

Results for an unusual test system of exposed caiman eggs are reported by [REDACTED] 2009, ASB2012-12002. Eggs were topically exposed in a laboratory setting to Roundup Full II GBF, and erythrocyte micronucleus formation was measured in hatchlings ([REDACTED] 2009, ASB2012-12002). The GBF tested was reported to contain the potassium salt of

glyphosate and alkoxyated alkylamine derivatives as surfactants. Statistically significant increases in micronucleated erythrocytes were observed in hatchlings from eggs treated with 500-1750 µg/egg. This system is quite unusual in the species tested and even more so in using an egg application with measurement of effects in hatchlings. Although there is some experience with a hen's egg erythrocyte micronucleus assay using *in ovo* exposure the erythrocytes are evaluated in embryos with only a few days between treatment and the erythrocyte micronucleus end point. In the reported caiman egg assay there was presumably a single topical exposure followed by an egg incubation period of about 10 weeks before hatching. Biological plausibility raises questions whether genotoxic events *in ovo* can produce elevated micronucleated erythrocyte frequencies detectable after 10 weeks, given the number of cell divisions occurring in development of a hatchling.

One published study reported a weak positive result in a *Drosophila* wing spot assay (Kaya et al., 2000, ASB2013-9832). Statistically significant positive increases were only in one of four crosses for small twin spots and not for the two other wing spot categories (large wing spots and twin wing spots). As discussed above, only negative or inconclusive results were observed for crosses that were not subject to mitotic recombination effects. If the result was actually treatment related it only would indicate an increase in recombination events and not in somatic mutations.

The above *in vivo* chromosome effect assays in non-mammalian systems give discordant results for reasons that aren't precisely defined. Typically these results would be given lower weight than mammalian systems in being predictive of mammalian effects, especially since there is little or practically no assay experience with these systems in comparison with *in vivo* mammalian chromosome effects assays, such as the rat or mouse bone marrow chromosome aberration or erythrocyte micronucleus assays.

B.6.4.8.6 DNA damage and other end points

A number of studies of glyphosate and GBFs have been published since 2000 which used various DNA damage end points in a variety of *in vitro* and *in vivo* systems. The DNA damage category includes end points such as sister chromatid exchange and DNA repair response in bacteria, but the most common DNA damage end point encountered was the alkaline single cell gel electrophoresis end point (alkaline SCGE) also commonly referred to as the "comet" assay. The alkaline SCGE end point has been applied to both *in vitro* and *in vivo* test systems.

In addition to DNA damage there are a few reports of other types of studies which can be associated with genotoxic effects even though the end points are not specific indicators of genotoxicity per se. These include sperm morphology and carcinogenicity studies.

***In vitro* DNA Damage Studies**

Some positive results for glyphosate or GBFs in the SCE end point were reported in cultured human and bovine lymphocytes in the earlier review (Williams et al., 2000, ASB2012-12053). These results tended to be weak, inconsistent and with limited evidence for dose response. A number of limitations were observed for the studies such as the failure to control pH and abnormally low control values. Additional *in vitro* DNA damage end point results described in the earlier review included negative results for glyphosate in the *B. subtilis* rec-assay and in the primary hepatocyte rat hepatocyte unscheduled DNA synthesis assay.

There are two subsequent publications using *in vitro* cultured mammalian cells and the SCE endpoint. Positive SCE results were reported for the uncharacterised herbazid GBF in mouse spleen cells (Amer et al., 2006, ASB2012-11539). The dose response pattern for SCE

response in this study was similar to the response for chromosome aberrations in this publication. Limitations of this study are in common to those described above for the chromosome aberration end point portion of the study; no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Positive SCE results were also reported for cultured bovine lymphocytes treated with up to 1.12 mM glyphosate for 24 and 48 hours without exogenous mammalian metabolic activation (Sivikova and Dianovsky, 2006, ASB2012-12029). The highest dose of 1.12 mM significantly delayed cell cycle progression with 48 hour treatment. These same concentrations for 24 h exposures did not induce statistically significant increases in chromosome aberrations which provides a clear example of a differential response of the SCE endpoint (Sivikova and Dianovsky, 2006, ASB2012-12029). This is an important consideration in these publications, as chromosome effects are considered more relevant to genotoxicity than DNA damage. Positive results for glyphosate are reported for the alkaline SCGE end point in three publications. Positive SCGE results were observed for two mammalian cell lines exposed to glyphosate for 4 hours at concentrations of 4.5-6.5 mM (GM39 cells) and 4.75-6.5 mM (HT1080 cells) (Monroy et al., 2005, ASB2012-11910). These concentrations are close to the upper limit dose of 10 mM generally recommended for *in vitro* mammalian cell assays and control of medium pH is not indicated. Characterisation of nuclear damage was done by visual scoring without coding of slides being indicated. Positive alkaline SCGE results were also reported in Hep-2 cells exposed for 4 hours to 3.5-7.5 mM glyphosate (Manas et al., 2009, ASB2012-11892). Higher concentrations of glyphosate were reported to result in viability of <80 % as determined by dye exclusion. As noted for the preceding publication, the concentrations employed were reasonably close to a limit dose of 10 mM and control of medium pH was not reported. This publication reported negative results for the chromosome aberration endpoint in cultured human lymphocytes exposed to up to 6 mM glyphosate for 48 hours and it should be noted that in this case an appropriate control of medium pH was reported for this human lymphocyte experiment. Positive alkaline SCGE results have also been reported for cultured human lymphocytes exposed to glyphosate at concentrations up to 580 µg/ml (estimated 3.4 mM) for 4 hours (Mladinic et al., 2009, ASB2012-11906). Effects were observed both in the presence and absence of S9 with statistically significant increases in tail intensity at 3.5, 92.8 and 580 µg/ml without S9 and at 580 µg/ml with S9. A modification of the alkaline SCGE assay employing human 8-hydroxyguanine DNA-glycosylase (hOGG1) to detect oxidative damage only indicated statistically significant effects on tail length for treatment with 580 µg/ml with S9. Increases in nuclear abnormalities (nuclear buds and/or nucleoplasmic bridges) were also observed at 580 µg/mL with and without S9 and in micronucleus frequency at 580 µg/ml with S9. Measurements of total antioxidant capacity and thiobarbituric acid reactive substances showed statistically significant increases at 580 µg/ml in the presence or absence of S9. Interpretation of the significance of metabolic activation effects is complicated by the observation that several of the end points (alkaline SCGE tail intensity and nuclear abnormalities) tended to show increases in the presence of S9 in negative controls or at the very lowest concentrations of glyphosate. A reasonable summation of the results in this publication is that alkaline SCGE effects and other effects such as nuclear abnormalities, early apoptosis, necrosis and oxidative damage were consistently observed at 580 µg/mL.

In addition to mammalian cell studies there are publications reporting positive alkaline SCGE effects for glyphosate in *Tradescantia* flowers and nuclei exposed to up to 700 µM glyphosate (Alvarez-Moya et al., 2011, ASB2012-11538) and in the *E. coli* SOS chromotest for DNA damage conducted on a Roundup BIO GBF (Raipulis et al., 2009, ASB2012-12008).

Observations of DNA damage in plants exposed to glyphosate are of questionable significance because of the herbicidal nature of glyphosate and the SOS chromotest provides only indirect evidence of DNA damage in a bacterial system.

Overall there appear to be a number of studies in which glyphosate or GBFs have been reported to produce positive responses in DNA damage endpoints of SCE or alkaline SCGE *in vitro* in mammalian cells. Most of these have occurred with exposures to mM concentrations of glyphosate. Although this dose level range is lower than the limit dose of 10 mM recommended for several *in vitro* mammalian cell culture assays (OECD473, 1997; OECD476, 1997; OECD487, 2010), an even lower limit dose of 1 mM was recently recommended for human pharmaceuticals, particularly because of concerns about relevance of positive *in vitro* findings observed at higher dose levels. In addition, many of the studies have limitations such as not indicating control of medium pH and not coding slides for visual scoring.

Concerns over the possibility of effects induced by toxicity have led to several suggestions for experimental and interpretive criteria to distinguish between genotoxic DNA-reactive mechanisms for induction of alkaline SCGE effects and cytotoxic or apoptotic mechanisms. One recommendation for the *in vitro* alkaline SCGE assay is to limit toxicity to no more than a 30 % reduction in viability compared to controls. Importantly, dye exclusion measurements of cell membrane integrity, such as those reported in some of the above publications may significantly underestimate cytotoxicity that could lead to alkaline SCGE effects. Other recommendations include conducting experiments to measure DNA double strand breaks to determine if apoptotic process might be responsible for alkaline SCGE effects. Measurement of apoptotic and necrotic incidence were only performed in one publication (Mladinic et al., 2009, ASB2012-11906) and these measurements indicated both apoptotic and necrotic processes occurring in parallel with observations of alkaline SCGE effects. These direct observations as well as the reported dose responses, consistently suggest that biological effects and cytotoxicity accompany the observations of DNA damage *in vitro* in mammalian cells and therefore confirm the likelihood that the observed effects are secondary to cytotoxicity and are thresholded.

***In vivo* DNA damage studies**

In the earlier review positive results for DNA strand breakage were reported for mice treated by the i.p. route with glyphosate and GBFs and for the alkaline SCGE endpoint in tadpoles of the frog *Rana catesbiana* exposed to a GBF (██████████, 2000, ASB2012-12053).

Only one *in vivo* mammalian DNA damage study was since reported. This report indicated an increase in SCE frequency in bone marrow cells of mice treated with uncharacterised herbicide GBF (██████████ 2006, ASB2012-11539). Statistically significant positive effects were only observed at the highest dose level tested (200 mg/kg administered p.o.).

Several recent publications report alkaline SCGE results for GBFs in aquatic species. Three publications reported positive alkaline SCGE results in aquatic vertebrates exposed to Roundup GBFs in water. These publications have a common feature that alkaline SCGE results were reported as visually scored damage category incidence rather than instrumental measurements of properties such as the tail length or tail intensity. In one publication increases in nuclei exhibiting alkaline SCGE visual damage effects were observed in erythrocytes and gill cells of the tropical fish *Prochilodus lineatus* exposed to 10 mg/litre of a Roundup GBF in water (██████████ 2008, ASB2012-11586). Results were variable with cell type and incubation; statistically significant positive responses were observed for

erythrocytes at 6 hours and 96 hours, but not 24 hours or for branchial cells from the gills at 6 hours and 24 hours. Measurement of erythrocyte micronucleus frequency and nuclear abnormalities did not show statistically significant increases in these endpoints. The concentration used was reported to be 75 % of the 96 hour LC₅₀, but trypan blue dye measurements apparently indicated >80 % viability of cells used in the alkaline SCGE assays. A second publication reported positive alkaline SCGE results in erythrocytes of the goldfish, *Carassius auratus*, exposed to 5, 10 and 15 ppm of a Roundup GBF for 2, 4 or 6 days (█ 2007, ASB2012-11587). Similar effects were observed for other end points (micronucleus and nuclear abnormalities). In general, effects increased with concentration and time. This publication did not report toxicity measurements or, more specifically, measurements of cell viability in the population studied. Positive results were also reported in erythrocytes of the European eel, *Anguilla anguilla*, exposed to 58 and 116 µg/liter of a Roundup GBF in water for 1 or 3 days (█ 2010, ASB2012-11836). Increases in nuclear abnormalities were also observed in erythrocytes from animals exposed for 3 days. Measurement of toxicity was not reported for the animals or erythrocytes; however, several endpoints relevant to antioxidant responses and oxidant effects were made in whole blood samples. No statistically significant effects were observed for catalase, glutathione transferase, glutathione peroxidase, glutathione reductase or reduced glutathione content. A large statistically significant increase for thiobarbituric acid reactive substances (TBARS, a measure of lipid peroxidation) was observed for the 115 µg/litre concentration group at 1 day. Statistically significant TBARS increases were not observed at 3 days, but, the 3-day negative control value appeared to be several fold higher than the 1-day value.

Significance of DNA damage end point results

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. It is widely recognised that *in vitro* DNA damage endpoints such as the SCE or alkaline SCGE can be induced by cytotoxicity and cell death processes rather than from DNA-reactive mechanisms.

The observation of effects of sodium dodecyl sulfate is also interesting because it suggests responses to surfactants which are typically components of GBFs. As a more specific example, polyoxyethylenealkylamine (POEA), a surfactant component of some GBFs has been shown to elicit cytotoxic effects such as perturbation of the mitochondrial membrane and disruption of mitochondrial membrane potential in cultured mammalian cells (█ 2007, ASB2009-9030). Surfactant effects provide a very plausible mechanism for observations of GBFs inducing DNA damage responses. Such responses would be expected to be associated with cytotoxicity-inducing exposures and exhibit a threshold.

With the exception of a mouse bone marrow SCE effect of herbazed GBF all of the reported *in vivo* DNA damage results are in non-mammalian species with limited experience and none of the assays reported evaluations for cytotoxic effects recommended for *in vivo* assays.

B.6.4.8.7 Human and environmental studies

A number of human and environmental studies have been published in or after 2000 where some exposures to GBFs in the studied populations were postulated. These publications are summarised in Table B.6.4-29.

Table B.6.4-29: Studies of Human and Environmental Populations with Reported or Assumed Glyphosate Exposure

Exposed Population	End point	Exposures	Result	Reference
<i>Human Studies</i>				
Open field and fruit farmers	Bulky DNA adducts	glyphosate formulation use reported in only 1 of 29 fruit farmers	No effects attributed to glyphosate formulation exposure	██████████ 2007 (ASB2012-11543)
Humans in areas where glyphosate formulation is applied	Lymphocyte cytokinesis block micronucleus (CB MN)	Aerial or manual spraying of glyphosate formulation for illicit crop control and sugar cane maturation	Increase in CB MN but no clear relationship to assumed or reported exposures	Bologne ██████████ 2009 (ASB2012-11570)
Floriculturists	Lymphocyte CB MN	Glyphosate formulation use reported in 21/51 workers with average of 106.5 kg applied	Increase in CB MN but not statistically significant	██████████ 2004 (ASB2012-11572)
Floriculturists	Lymphocyte CB MN	Glyphosate formulation use reported in 57/107 workers. Numerous other pesticides reported as used by a similar number or more of workers	Statistically significant increase in CB MN	██████████ 2002 (ASB2012-11573)
Agricultural workers	Buccal cell micronucleus	Glyphosate formulation use reported along with numerous other pesticides	Statistically significant increase in MN	██████████ 2009 (ASB2012-11570)
Fruit growers	Lymphocyte Alkaline SCGE; Ames test on urine	Glyphosate use reported in 2/19 1 day before captan spraying and 1/19 on the day of captan spraying	No effects attributable to glyphosate formulation exposure	██████████ 2003 (ASB2012-11878)
Agricultural workers	Lymphocyte CB MN; buccal cell micronucleus	Glyphosate formulation use reported in 16% of one of four populations studied (Hungary)	No statistically significant increases in CB MN or buccal cell micronucleus frequencies	██████████ 2003 (ASB2012-11991)
Individuals on or near glyphosate spraying	Lymphocyte alkaline SCGE	Glyphosate formulation aerially sprayed within 3 km	Statistically significant increases in damaged cells	██████████ 2007 (ASB2012-11992)
Greenhouse Farmers	Lymphocyte SCE	Glyphosate formulation use reported in 99/102 workers; numerous other pesticides used	Statistically significant increases in SCE	██████████ 2001 (ASB2012-12025)

Exposed Population	End point	Exposures	Result	Reference
Farmers	Lymphocyte CB MN	Glyphosate formulation use reported in 3/11 farmers	Statistically significant increase in micronucleus frequency but not in frequency of binucleated cells with micronuclei	██████████ 2006 (ASB2012-12045)
<i>Environmental Studies</i>				
Meadow voles living on golf courses	Blood cell alkaline SCGE; erythrocyte micronucleus	Glyphosate formulation use reported along with numerous other pesticides	Some effects judged possibly related to Daconil® fungicide	██████████ 2004 (ASB2012-11871)
Fish from dams (various species)	Erythrocyte micronucleus	Glyphosate formulation use reported in adjacent lands along with other pesticides	Higher MN frequencies than normal or expected but no negative concurrent controls used	██████████ 2011 (ASB2012-12017)

Many of the human studies either found no effects attributable to GBFs or the reported GBF usage by the studied population was too low to be associated with observed population effects (██████████ 2007, ASB2012-11543; ██████████ 2004, ASB2012-11572; ██████████, 2003, ASB2012-11878; ██████████, 2003, ASB2012-11991; ██████████ 2006, ASB2012-12045).

In some studies, incidence of GBF use by the population studied was significant but high incidence of use of other pesticides was also reported (██████████, 2002, ASB2012-11573; ██████████, 2001, ASB2012-12025). Even though positive effects were observed in these populations, ascribing these effects to any particular environmental exposure is not scientifically justifiable and such results certainly cannot be considered as definitive evidence for GBF-induced human genotoxic effects.

Two published studies focused on populations believed to be exposed to GBFs by their presence at or near aerial or manual spraying operations. One publication reported induction of alkaline SCGE effects in blood lymphocytes of populations living within 3 km of areas sprayed with glyphosate formulation for illicit crop eradication (██████████ 2007, ASB2012-11992). The populations studied were relatively small (24 exposed individuals and 21 non-exposed individuals). The sprayed material was reported to be Roundup Ultra, a GBF containing 43.9 % glyphosate, polyethoxylated ██████████ surfactant and a proprietary component, Cosmoflux 411F. Specific methods for collection, storage, and transport of blood samples are not described for either the exposed population or control group. The publication also does not indicate that slides were coded for scoring which consisted of visual classification into damage categories and measurement of DNA migration (tail length). There were fairly large differences in ages and sex distribution of the exposed and control populations but these did not appear to be statistically significant. The study reported increases in damaged cell categories and statistically significant increases in DNA migration (tail length) in the presumably exposed population. Interpretation of the results of this study should consider numerous reported signs of toxicity in the exposed population and the reported application rate of 24.3 litres/ha which was stated to be 20 times the maximum recommended application rate. Some of the reported human health effects described by ██████████ (2007, ASB2012-11992) appear to be consistent with severe exposures noted in clinical reports of acute poisoning incidents with GBFs and other pesticide formulations

(often self-administered) rather than typical bystander exposures. Given the considerably favorable general toxicology profile of glyphosate as reported by the WHO/FAO Joint Meeting on Pesticide Residues (WHO/FAO, 2004, ASB2008-6266) and in [REDACTED] (2000, ASB2012-12053), factors related to either high surfactant exposure, unusual GBF components in this formulation or other undocumented variables appear to be confounding factors in this study. It appears that the reported alkaline SCGE effects could well have been secondary to the ailments reported in this study population.

A second publication reported results for a blood lymphocyte cytokinesis-block micronucleus study of individuals in areas treated with glyphosate formulation by aerial spraying or manual application [REDACTED] 2009, ASB2012-11570). Although the title of the publication contains the term “agricultural workers”, most of the populations studied do not appear to be agricultural workers who are involved in application of GBFs. The human lymphocyte culture and scoring methodology employed in the [REDACTED] (2009, ASB2012-11570) study appear to be generally consistent with commonly used and recommended practices for this assay. However, there is a significant question as to how long the blood samples used in the study were stored prior to initiating cultures and this may have affected the micronucleus numbers observed in the different sets of samples and populations. Also, the populations in the aerially sprayed regions had a second sampling a few days after the first sampling and this second sampling was not performed in the control populations. The publication reported a small increase in the frequency of binucleated cells with micronuclei and micronuclei per cell in samples collected from people living in three regions after spraying of GBFs compared with control values of samples collected just before spraying. However, the pattern of the increases did not correlate either with the application rate or with self-reported exposure. The largest post-spraying increase in binucleated cell micronucleus frequency was reported for a population with a much lower glyphosate active ingredient application rate and only 1 of 25 people in this region reported contact with sprayed glyphosate formulation. Increases in binucleated cell micronucleus frequency did not have a statistically significant relationship with self-reported exposure for two other populations. Some interpretative statements in [REDACTED] (2009, ASB2012-11570) suggest a small transient genotoxic effect of glyphosate formulation spraying on frequencies of binucleated cells with micronuclei, but other statements indicate that causality of the observed effects could not be determined using reasonable criteria and that lack of exposure data precluded conclusions. This study has a combination of uncontrolled or inadequately characterized variables, such as uncharacterised exposure to “genotoxic pesticides”, that would appear to preclude using the data to support any conclusion that exposure to GBFs affects binucleated micronucleus frequencies. Actually, the available data, while certainly limited in nature, support a conclusion that the observed effects do not appear to be attributable to glyphosate formulation exposure. This conclusion is reinforced by [REDACTED] (2004, ASB2012-11528), where biomonitoring of agricultural workers applying GBFs reports systemic exposures orders of magnitude below *in vivo* model chromosome aberration and micronucleus study doses, the majority of which were negative for glyphosate and GBFs.

There are two publications related to environmental monitoring for genotoxic endpoints. One study using blood cell alkaline SCGE and micronucleus endpoints was conducted on samples from meadow voles living on or near golf courses where pesticides had been applied (Kne [REDACTED] 2004, ASB2012-11871). Results were significantly inconsistent between two seasons. Although some suggestions of effects were reported, glyphosate was only one of a number of applied pesticides and the effects observed were considered as possibly attributable to exposure to Daconil® fungicide. A second publication reported results for the erythrocyte micronucleus assay applied to fish collected from several dams in Brazil ([REDACTED] 2011, ASB2012-12017). Glyphosate formulation was one of a number of

pesticides reported to be used in the area of the dams. No efforts appear to have been made to measure glyphosate or other pesticide concentrations in any of the ten dams from which fish were sampled. This study reported what were considered to be high levels of micronucleated cell frequency but there were no concurrent negative controls. In the absence of these controls the results cannot be interpreted as indicating any effect of pesticide exposure.

Although there have been a fairly large number of human genotoxicity studies reported where there was some exposure to GBFs, the large majority of these studies do not allow any conclusions about possible effects of glyphosate or GBFs because the exposure incidence was low or because there were reported exposures to a large number of pesticides. One report found an increase in alkaline SCGE effects in humans living in or near areas where a GBF was sprayed but that study had a number of methodology reporting and conduct deficiencies and the reported effects could well have been due to toxicity reported in the study population. A second study found some increases in cytokinesis-block micronucleus frequency in humans possibly exposed to GBFs but the effects were not concordant with application rates or self-reported exposures and thus do not constitute reliable indications of effects for this endpoint in humans exposed to GBFs. Neither of the two environmental monitoring studies in meadow voles or fish provide any reliable evidence of exposures to glyphosate or GBFs or adverse effects resulting from potential exposures to glyphosate or GBFs.

B.6.4.8.8 DNA-Reactivity and carcinogenesis

As noted in the earlier review, ³²P-postlabelling DNA adduct studies in mice did not indicate formation of adducts from glyphosate and questionable evidence of adducts from Roundup GBF administered as a high 600 mg/kg i.p. dose in an unusual dimethylsulfoxide/olive oil vehicle (1998, TOX1999-318; 2000, ASB2012-12053). Another earlier reviewed study reported DNA strand breakage in liver and kidneys of mice injected i.p. with glyphosate and Roundup GBF. This study also reported an increase in 8-hydroxydeoxyguanosine (8-OHdG) residues in liver DNA from mice injected with glyphosate but not GBF. Increased 8-OHdG was found in kidney DNA from mice injected with GBF but not glyphosate (1997, Z59299; 2000, ASB2012-12053). No new direct studies of DNA reactivity of glyphosate or GBFs were encountered in publications since 2000. One publication did report on studies in mice to further investigate toxic effects and 8-OHdG levels associated with the routes, vehicles and dose levels employed in earlier ³²P-postlabelling and DNA strand breakage and 8-OHdG studies (2008, ASB2012-11845). This publication reported that high i.p. dose levels of GBF induced significant liver and kidney toxicity that were not observed with oral administration. Statistically significant increases in 8-OHdG were not observed in this study under the same conditions as employed by the earlier study. The dimethylsulfoxide/olive oil vehicle dramatically enhanced toxicity of GBF administered by the i.p. route and the toxicity was also observed for formulation components without glyphosate. These results indicated that the effects reported in the earlier studies were associated with high liver and kidney toxicity that was primarily due to the non-glyphosate components of the formulation and which were produced by the i.p. route of exposure to very high dose levels. The enhancement of toxicity by the unusual dimethylsulfoxide/olive oil dosing vehicle further calls into question whether the ³²P-postlabelling finding represented effects associated with unusual toxicity rather than being indicative of adducts formed from glyphosate or glyphosate formulation components.

Carcinogenicity is not a direct endpoint for genotoxicity but it is one of the possible consequences of genotoxicity and, conversely, lack of carcinogenicity in well-conducted experimental studies provides some evidence that a significant genotoxic mode of action is not operating *in vivo*. The earlier review of glyphosate concluded that it was not carcinogenic in mouse or rat chronic studies and notes that glyphosate was not considered carcinogenic by numerous regulatory agencies and scientific organisations (█ 2000, ASB2012-12053).

B.6.4.8.9 AMPA and POEA

In addition to glyphosate and GBFs, the earlier review included information on the toxicity and genotoxicity of the major environmental breakdown product of glyphosate, aminomethylphosphonic acid (AMPA), and what was at that time a common GBF surfactant mixture of polyethoxylated long chain alkylamines synthesized from animal-derived fatty acids (polyethoxylated tallow amine, █ ethoxylate, POEA). Today a wide variety of surfactant systems are employed by different companies for different regions and end uses.

In the earlier review, summarised genotoxicity results for AMPA included negative results in the Ames/*Salmonella* bacterial reversion assay, an *in vitro* unscheduled DNA synthesis assay in primary hepatocytes and a mouse bone marrow erythrocyte micronucleus assay (█ 2000, ASB2012-12053). One publication of AMPA genotoxicity results was observed subsequent to 2000. In this publication analytical grade AMPA was reported to have positive effects in several assays including an alkaline SCGE endpoint in cultured mammalian Hep-2 cells, a chromosome aberration endpoint in cultured human lymphocytes and in a mouse bone marrow erythrocyte micronucleus assay (█ 2009, ASB2012-11891). Experimental limitations in the conduct of the alkaline SCGE assay included no inclusion of mammalian metabolic activation and no reported control of medium pH even though relatively high concentrations of AMPA acid (2.5-10 mM for 4 hours) were used. Although nucleoid images were analyzed with software rather than visual analysis the methodology doesn't indicate that slides were coded and there may have been a visual judgment component in selection of images for analysis. The positive results were statistically significant increases in tail length, % DNA in tail and tail moment at 4.5 to 7.5 mM AMPA. The human lymphocyte chromosome aberration assay also did not employ an exogenous mammalian metabolic activation system but control of medium pH and blind scoring of slides were reported for this assay. A small increase in chromosome aberrations per 100 metaphases was observed in cells exposed to 1.8 but not 0.9 mM AMPA for 48 hours. The increase was marginally significant ($p < 0.05$) and no statistically significant increases were observed for any specific chromosome aberration category. Although number of cells with aberrations are commonly used to describe results from *in vitro* chromosome aberration assays (OECD473, 1997) these data were not presented. Given the marginal significance, these omissions are a significant limitation in interpreting the results. Positive results were also reported for a mouse micronucleus bone marrow assay in mice administered 2 x 100 mg/kg or 2 x 200 mg/kg i.p at 24 hour intervals. The methodology description did not indicate that slides were coded for analysis in this assay. Results were reported as a statistically significant increase from a negative control value of 3.8/1000 micronucleated erythrocytes to 10.0 and 10.4/1000 micronucleated erythrocytes in the 2 x 100 and 2 x 200 mg/kg dose groups, respectively. These data do not indicate a reasonable dose response and a third dose level was not employed as recommended for this assay (OECD474, 1997). The publication indicates micronucleus scoring results for "erythrocytes" and not polychromatic or immature

erythrocytes as would be appropriate for the acute dose protocol employed. Although this might be an inadvertent error in methodology description the term polychromatic erythrocytes was used in the methods section and PCE was used in the results table to describe scoring of PCE/NCE ratio.

The reported positive effects for AMPA in the *in vitro* studies are not concordant with *in vitro* results for other endpoints or the lack of genotoxic structural alerts in the structurally similar parent molecule moieties from DEREK *in silico* analysis. The alkaline SCGE effect could be due to cytotoxicity, especially considering the relatively high dose levels employed (close to the 10 mM upper limit dose) and the lack of indication of pH control. Although limited cytotoxicity (>80 % viability) was reported using the trypan blue exclusion method this endpoint may grossly underestimate cytotoxic effects observed with other end points.

The *in vitro* chromosome aberration assay positive result was of low magnitude and was of particularly questionable significance, considering the lack of statistical significance for any individual chromosome aberration category and that the results for number or percent of cells with chromosome aberrations were not reported.

There is a clear discordance in results for AMPA in the mouse bone marrow micronucleus assay. In the earlier review negative results were reported for AMPA in a mouse bone marrow micronucleus assay conducted with dose levels up to 1000 mg/kg i.p. (Williams et al., 2000, ASB2012-12053). The maximum dose level was much higher than those used by Williams et al. (2009, ASB2012-11891). Although Williams et al. used a protocol with two doses separated by 24 hours and a single harvest time, this protocol would not be expected to give different results than a single dose with multiple harvest times, particularly when the maximum single dose was much higher (OECD 474, 1997). PCE/NCE ratio data from the Williams et al. (2009, ASB2012-11891) study do not indicate that there were detectable bone marrow toxic effects observed under the conditions of their study. It appears possible that Manas et al. may have inappropriately scored erythrocytes for micronuclei instead of polychromatic erythrocytes, but if this is the case lower sensitivity rather than higher sensitivity would be expected. These limitations suggest the possibility that the aberrant result might be that of Williams et al. (2009, ASB2012-11891) but further studies might be necessary to resolve the discordance.

The earlier review reported negative results for POEA in an Ames/*Salmonella* assay (Williams et al., 2000, ASB2012-12053). No other genotoxicity results were reported for POEA individually but numerous genotoxicity results were presented, as described earlier, for GBFs containing POEA. Examination of subsequent literature for this review did not produce any new publications reporting genotoxicity results for POEA as an individual test material (i.e. not as a glyphosate formulation). However, there were some publications confirming that POEA can be a significant contributor to toxicity of GBFs and that it exhibits biological effects consistent with surfactant properties. As noted earlier, experiments with a POEA-containing formulation without glyphosate administered i.p. in DMSO/olive oil vehicle to mice produced the same severe liver and kidney toxicity as a GBF indicating that the toxicity primarily resulted from the formulation components rather than glyphosate (Heydens et al., 2008, ASB2012-11845). Similarly, dose-response curves were superimposed for an *in vitro* system evaluating a GBF and the same formulation without glyphosate present (Levine et al., 2007, ASB2009-9030). Effects on mammalian cells consistent with membrane disruption and consequent cytotoxicity were observed for POEA (Benachour and Seralini, 2009, ASB2012-11561).

B.6.4.8.10 Genotoxicity Weight of Evidence

The earlier review applied a weight of evidence analysis to the available genotoxicity data. Various weighted components included assay system validation, test system species, relevance of the endpoint to heritable mutation, reproducibility and consistency of effects and dose-response and relationship of effects to toxicity (2000, ASB2012-12053). The conclusion of this analysis was that glyphosate and Roundup GBFs were not mutagenic or genotoxic as a consequence of direct chemical reaction with DNA. This was supported by a strong preponderance of results indicating no effects in *in vivo* mammalian assays for chromosome effects and consistently negative results in gene mutation assays. Although some DNA damage responses were noted, these were judged likely to be secondary to toxicity rather than DNA reactivity.

Since this earlier review, a large number of genotoxicity studies have been conducted with glyphosate and GBFs. For gene mutation, one of the two primary endpoint categories with direct relevance to heritable mutation, one subsequent publication contains a summary of results from a bacterial gene mutation endpoint assay (Ames/*Salmonella* bacterial reversion assay). Although there were very significant limitations to the information published, the negative result is consistent with the majority of negative results reported for glyphosate and GBFs in Ames/*Salmonella* bacterial reversion assays. Another publication reported results for a *Drosophila* wing spot assay of glyphosate. Results were negative or inconclusive in this assay for crosses that would have detected gene mutation as loss of heterozygosity. The new results provide some support to reinforce the earlier conclusion that glyphosate and GBFs are not active for the gene mutation endpoint category.

The second primary endpoint category with direct relevance to heritable mutation is chromosome effects. The earlier review noted mixed results for two *in vitro* chromosome effects assays in mammalian cells but concluded that the most reliable result was the negative assay. A number of *in vitro* mammalian cell chromosome aberration or micronucleus assay results have been subsequently published using bovine or human lymphocytes. These assays suffer from some technical limitations in conduct or reporting of methodology that frequently included failure to indicate control of medium for pH and failure to indicate coding of slides for visual scoring. Both positive and negative results are reported in these assays. A large preponderance of results in the absence of an exogenous mammalian metabolic activation system were negative up to high (mM) dose levels that were toxic or close to toxic levels observed in parallel experiments. The exceptions were a weak and inconsistent response reported in two publications from the same laboratory and a positive response for the uncharacterized formulation, herbazed. In addition to these findings in mammalian cells negative results were also reported for Roundup GBF in an onion root tip assay conducted without exogenous mammalian metabolic activation. Thus, the preponderance of evidence from assays not employing an exogenous mammalian metabolic activation system indicates that glyphosate and GBFs are not structural chromosome breakage inducers (clastogenic) in *in vitro* mammalian chromosome aberration or micronucleus assays.

Two publications from one laboratory reported an increase in micronucleus frequencies for glyphosate in *in vitro* cultured mammalian cells in the presence of an exogenous S9 metabolic activation system (Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012-11907). An enrichment for centromeric-containing micronuclei suggested that the increased micronuclei observed in these studies were derived from aneugenic processes, probably mediated through toxicity, rather than chromosome breakage. Thus, these two reports of weak micronucleus responses in the presence of exogenous mammalian metabolic activation appear to result from toxicity-associated aneugenic rather than clastogenic mechanisms. A number of other gene mutation and *in vitro* chromosome effect genotoxicity studies are negative with

exogenous metabolic activation which supports the conclusion that the weight of evidence does not indicate a DNA-reactive clastogenic activity in *in vitro* assays using mammalian cells.

All except one of a number of *in vivo* mouse bone marrow chromosome aberration or micronucleus assays of glyphosate and GBFs were reported as negative in the earlier review. In the updated review both positive and negative results were reported for glyphosate and GBFs in these types of assays. Many of these studies had limitations or deficiencies compared to international guidelines with the most common and significant being no indication of slide coding for visual scoring. Four publications from three laboratories reported negative results in mouse bone marrow erythrocyte micronucleus assays of glyphosate and GBFs which are consistent with the earlier reviewed studies. These studies used high, peri-lethal dose levels administered by the i.p. or oral routes.

Two publications from two laboratories reported positive results for glyphosate and GBFs in the mouse bone marrow erythrocyte micronucleus assay. One positive result for glyphosate was encountered using dose levels and routes that were similar to those employed in the negative glyphosate studies in the same assay system. The publication reporting this result indicates that erythrocytes rather than polychromatic erythrocytes were scored which would be inappropriate for the treatment protocol but it is possible that this is a misreporting of what types of cells were actually scored. Although there is no definitive explanation for the discordance, the preponderance of mouse bone marrow erythrocyte micronucleus studies of glyphosate are clearly negative. The reported positive result for Roundup GBF is discordant with a number of negative results for Roundup or other GBFs conducted at higher dose levels. The most unique feature of this study was the use of dimethylsulfoxide as a vehicle. The preponderance of mouse bone marrow erythrocyte micronucleus studies for Roundup and other GBF studies is negative.

Positive results were reported in an unusual test system (rabbit) and route (drinking water), but water intake was not reported and effects may therefore be attributable to dehydration. Furthermore, most of the effects were on endpoints not usually considered as indicators of clastogenicity and structural chromosome aberration. One laboratory reported positive results for chromosome aberration effects in bone marrow and spermatocytes after extended dosing. However, the herbazed formulation test material was not characterised.

While more discordant results in the important *in vivo* mammalian chromosome effect assay category have been reported in publications subsequent to the earlier 2000 review the preponderance of evidence continues to indicate that glyphosate and GBFs are not active in this category of endpoint.

Several *in vivo* erythrocyte micronucleus assay results for GBFs in non-mammalian systems (fish and caiman eggs) have been published since the earlier review. These test systems have relatively little experience and are largely unvalidated in comparison to the mouse bone marrow erythrocyte micronucleus assay. Two publications report negative results and two publications report positive results in different fish species and there is no definitive explanation for the discordance. Both the positive and negative studies employed maximum dose levels that were toxic or close to toxic dose levels. One possible explanation for the discordance is that the positive effects were associated with toxicity that only occurred beyond an exposure threshold and over a fairly narrow dose range. Positive results in hatchlings derived from caiman eggs exposed to Roundup formulation are given relatively little weight because of extremely limited experience with this assay system and because of significant questions about how DNA damage effects induced in embryos can persist and be evident in cells of hatchlings after several weeks and numerous cell divisions. The reported weak and inconsistent response in one of four crosses for somatic recombination in the *Drosophila* wing spot assay is also accorded relatively low weight. These non-mammalian

test systems are generally considered of lower weight for predicting mammalian effects than mammalian test systems. Also, the environmental significance of effects for GBFs should consider the relationship between concentrations or exposures producing effects and likely environmental concentrations or exposures. This is particularly important if the effects are produced by threshold mediated toxic processes.

There have been a significant number of publications since the earlier review of results for assays in the DNA damage category with some SCE and a large number of alkaline SCGE endpoint publications. In general, the DNA damage end point category is considered supplementary to the gene mutation and chromosome effect categories because this endpoint category does not directly measure heritable events or effects closely associated with heritable events. Regulatory genotoxicity testing recommendations and requirements focus on gene mutation and chromosome effect end points for initial core testing, particularly for *in vitro* testing. This consideration is underscored by the observation of some cases of compounds where positive effects are observed in these assays that are not observed for gene mutation or chromosome effect assays. Also, there are numerous examples of responses in these endpoints that do not appear to result from mechanisms of direct or metabolite DNA-reactivity. The unique response consideration is reinforced in this data set by observations of responses in DNA damage endpoints but not in chromosome effect end points.

Many DNA damage endpoint assays of glyphosate or GBFs have produced positive results at high, toxic or peri-toxic dose levels for the SCE and alkaline SCGE endpoints in a variety of test systems including cultured mammalian cells, several aquatic species and caiman eggs. The only new report of positive *in vivo* mammalian DNA damage effects are for an uncharacterised formulation, herbazed. There are several examples of negative results for a chromosome aberration or micronucleus endpoint and positive results for the alkaline SCGE or SCE endpoint in the same publication ([REDACTED] 2008, ASB2012-11586; [REDACTED] 2009, ASB2012-11892; [REDACTED] 2009, ASB2012-11906; [REDACTED] 2006, ASB2012-12029). These examples confirm the impression that the DNA damage endpoints are not necessarily predictive of heritable mutation effects and are also consistent with the DNA damage endpoints reflecting toxic effect mechanisms. While the number of reported positive responses in these endpoints does suggest that effects in these endpoints can be induced by glyphosate or GBFs, comparison with results for gene mutation and chromosome effects endpoints, examination of the dose response and association of the effects with toxic endpoints indicates that these effects are likely secondary to toxicity and are threshold mediated. Surfactants in GBFs increase toxicity compared to the active ingredient of glyphosate salts and are shown to induce effects such as membrane damage and oxidant stress which are likely capable of inducing DNA damage effects at cytotoxic doses. These factors as well as other considerations presented in Section 6.3 indicate that these DNA damage effects have negligible significance to prediction of hazard or risk at lower and more relevant exposure levels.

Most of the human studies do not provide interpretable or relevant information regarding whether there are *in vivo* human genotoxic effects of GBFs because the reported incidence of glyphosate formulation exposure in the population was low or because there were reported exposures to a relatively large number of pesticides. Two studies with focus on glyphosate exposure through presence in or near areas of glyphosate formulation spraying found increases in the DNA damage alkaline SCGE end point. In one study clinical signs of toxicity were reported in the population and spraying concentrations were reported to be many times the recommended application rate. Given the nature of the end point a reasonable interpretation is that effects might well be due to the overt toxicity that was reported in the publication. This would be a threshold mediated, non-DNA reactive mechanism and is consistent with experimental system results showing alkaline SCGE effects in animals

exposed to high levels of formulation components. The low weight of evidence for significant genotoxic hazard indicated by this particular endpoint in human monitoring is reinforced by findings that exercise induces alkaline SCGE effects in humans. The other study found increases in binucleated micronucleated cell frequency in population in spraying areas but the increases were not consistent with spraying levels or self-reported exposure. These latter observations are not consistent with the study presenting clear evidence of GBF effects on this endpoint. In sum, the available human data do not provide any clear indications that exposed humans are substantially different in response than mammalian animal models or that exposure to GBFs produces DNA-reactive genotoxicity.

Carcinogenicity is an adverse effect that is a possible consequence of genotoxic and mutagenic activity. Conversely, lack of carcinogenicity in properly conducted animal models is supportive for lack of significant *in vitro* mammalian genotoxicity. The updated review provides one new study of glyphosate formulation which is negative for either initiator or complete carcinogenesis activity which provides additional evidence to reinforce the conclusion from earlier mammalian carcinogenicity assays that glyphosate and GBFs are non-carcinogenic. These findings support the conclusion that glyphosate and GBFs do not have *in vivo* mammalian genotoxicity or mutagenicity.

In addition to considering the results relevant to genotoxicity hazard assessment, an important additional perspective on risk can be provided by comparing levels used in experimental studies with expected human and environmental exposure levels. A study of farmers indicated a maximum estimated systemic glyphosate dose of 0.004 mg/kg for application without protective equipment and a geometric mean of 0.0001 mg/kg (Acquavella et al., 2004, ASB2012-11528). When compared with *in vivo* mammalian test systems that utilize glyphosate exposures on the order of 50-300 mg/kg, the margins of exposure between the test systems and farmers is 12,500-75,000 for the maximum farmer systemic exposure and 0.5-3 million for the geometric mean farmer systemic exposure. These margins are quite substantial, especially considering that many of the *in vivo* genotoxicity studies are negative. Assuming reasonable proportionality between exposure to glyphosate and GBF ingredients, similar large margins of exposure would exist for GBF components. The margins of exposure compared to *in vitro* mammalian cell exposures are estimated to be even larger. Assuming uniform distribution, the systemic concentration of glyphosate from the Acquavella et al. (2004, ASB2012-11528) farmer biomonitoring study would be on the order of 24 nM for the maximum and 0.59 nM for the geometric mean exposure. A typical maximum *in vitro* mammalian exposure of 1-5 mM represents a margin of exposure of 42,000-211,000 for the maximum farmer exposure and 1.7-8.4 million for the geometric mean farmer systemic exposures, respectively.

Overall, the weight of evidence of the studies considered in the earlier review as well as the studies considered in this review indicates that glyphosate and GBFs are not genotoxic in the two general endpoint categories most directly relevant to heritable mutagenesis, gene mutation and chromosome effects. This conclusion results from a preponderance of evidence; however, there are reports of positive discordant results in both end point categories. The new studies considered in this review provide some evidence for DNA damage effects induced by high, toxic exposures, particularly for the alkaline SCGE end point and for GBFs containing surfactant. Several considerations, including the lack of response in other endpoint categories, suggest that these effects result from toxic and not DNA-reactive mechanisms and that they do not indicate *in vivo* genotoxic potential under normal exposure levels.

Regulatory and authoritative reviews of glyphosate supporting registrations and registrations in all regions of the world over the last 40 years have consistently determined that glyphosate is nongenotoxic (Commission, 2002, ASB2009-4191; WHO/FAO, 2004, ASB2008-6266). Scientific publications contrary to these regulatory reviews should be evaluated using a

weight of evidence approach with consideration for reliability of the assay used and data quality presented.

Abbreviations

AMPA, aminomethylphosphonic acid ; CB MN, cytokinesis block micronucleus; GBF, glyphosate based formulation; i.p., intraperitoneal ; NCE, normochromatic erythrocyte; OECD, Organization for Economic Cooperation and Development; PCE, polychromatic erythrocyte; POEA, polyethoxylated tallow amine, [REDACTED] ethoxylate; SCE, sister chromatid exchange; SCGE, single cell gel electrophoresis (comet).

Author(s)	Year	Study title
Alvarez-Moya, C., Silva, M.R., Arambula, A.R.V., Sandoval, A.I., Vasquez, H.C., Gonzales Montes, R.M.	2011	Evaluation of genetic damage induced by glyphosate isopropylamine salt using <i>Tradescantia</i> bioassays Genetics and Molecular Biology Volume: 34 Number: 1 Pages: 127-130 ASB2012-11538

Abstract*

Glyphosate is noted for being non-toxic in fishes, birds and mammals (including humans). Nevertheless, the degree of genotoxicity is seriously controversial. In this work, various concentrations of a glyphosate isopropylamine salt were tested using two methods of genotoxicity assaying, viz., the pink mutation assay with *Tradescantia* (4430) and the comet assay with nuclei from staminal cells of the same plant. Staminal nuclei were studied in two different forms, namely nuclei from exposed plants, and nuclei exposed directly. Using the pink mutation assay, isopropylamine induced a total or partial loss of color in staminal cells, a fundamental criterion utilised in this test. Consequently, its use is not recommended when studying genotoxicity with agents that produce pallid staminal cells. The comet assay system detected statistically significant ($p < 0.01$) genotoxic activity by isopropylamine, when compared to the negative control in both the nuclei of treated plants and directly treated nuclei, but only the treated nuclei showed a dose-dependent increase. Average migration in the nuclei of treated plants increased, when compared to that in treated nuclei. This was probably due, either to the permanence of isopropylamine in inflorescences, or to the presence of secondary metabolites. In conclusion, isopropylamine possesses strong genotoxic activity, but its detection can vary depending on the test systems used.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Exposure conditions of plants (immersion) not representative for glyphosate. Inappropriate test model as herbicides are toxic to plants. Presentation of results not sufficient for assessment. Reporting deficiencies (e.g. positive controls).

Relevance of study:

Not relevant (Due to reliability, and exposure conditions of plants and inappropriate test model).

Klimisch code:

3

Author(s)	Year	Study title
Bolognesi, C. Bonatti, S. Degan, P. Gallerani, E. Peluso, M. Rabboni, R. Roggieri, P. Abbondandolo, A.	1997	Genotoxic activity of glyphosate and its technical formulation roundup Journal of Agricultural and Food Chemistry Volume: 45 Pages: 1957-1962 Z59299

Abstract*

Glyphosate (N-phosphonomethylglycine) is an effective herbicide acting on the synthesis of aromatic amino acids in plants. The genotoxic potential of this herbicide has been studied: the results available in the open literature reveal a weak activity of the technical formulation. In this study, the formulated commercial product, Roundup, and its active agent, glyphosate, were tested in the same battery of assays for the induction of DNA damage and chromosomal effects *in vivo* and *in vitro*. Swiss CD1 mice were treated intraperitoneally with test substances, and the DNA damage was evaluated by alkaline elution technique and 8-hydroxydeoxyguanosine (8-OHdG) quantification in liver and kidney. The chromosomal damage of the two pesticide preparations was also evaluated *in vivo* in bone marrow of mice as micronuclei frequency and *in vitro* in human lymphocyte culture as SCE frequency. A DNA-damaging activity as DNA single-strand breaks and 8-OHdG and a significant increase in chromosomal alterations were observed with both substances *in vivo* and *in vitro*. A weak increment of the genotoxic activity was evident using the technical formulation.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Basic data given, however, the study is performed with methodological and reporting deficiencies (only data without metabolic activation generated in *in vitro* tests, no positive controls included in *in vitro* SCE and *in vivo* experiments, in some experiments only two test substance concentrations tested).

Relevance of study:

Not relevant (Due to methodological and reporting deficiencies data considered to be supplemental information; i.p. exposure route is not relevant for human exposure)

Klimisch code:

3

Author(s)	Year	Study title
Bolognesi, C., Perrone, E., Landini, E.	2002	Micronucleus monitoring of a floriculturist population from western Liguria, Italy Mutagenesis Volume: 17 Number: 5 Pages: 391-397 ASB2012-11573

Abstract*

A biomonitoring study was carried out to investigate whether exposure to complex pesticide mixtures in ornamental crop production represents a potential genotoxic risk. Exposed and control subjects were selected in western Liguria (Italy). The area was chosen for its intensive use of pesticides. The main crops produced were roses, mimosas, carnations and chrysanthemums, as ornamental non-edible plants, and tomato, lettuce and basil, as edible ones. The levels of micronuclei (MN) were analysed in peripheral blood lymphocytes of 107 floriculturists (92 men and 15 women) and 61 control subjects (42 men and 19 women). A statistically significant increase in binucleated cells with micronuclei (BNMN) was detected in floriculturists with respect to the control population (4.41 +/- 2.14 MN/1000 cells versus 3.04 +/- 2.14, $P < 0.001$). The mean number of BNMN varied as a function of sex and age. Smoking habit had no effect on MN frequency. A positive correlation between years of farming and MN frequency in peripheral blood lymphocytes was observed ($r = 0.30$, $P = 0.02$). The conditions of exposure were also associated with an increase in cytogenetic damage, with a 28 % higher MN frequency in greenhouse workers compared with subjects working only outdoors in fields. Workers not using protective measures during high exposure activities showed an increase in MN frequency. Our findings suggest a potential genotoxic risk due to pesticide exposure.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable for glyphosate

Comment:

MN-test comparable to OECD guidelines, but not equal. Exposures to multiple pesticides with no information on exposure concentrations to individual pesticides make result unreliable for glyphosate.

Relevance of study:

Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenicity possible. Not relevant for glyphosate).

Klimisch code:

3

Author(s)	Year	Study title
Bolognesi, C., Landini, E., Perrone, E., Roggieri, P.	2004	Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centromeric probe Mutation Research Volume: 557 Number: 2 Pages: 109-117 ASB2012-11572

Abstract*

Flower production in greenhouses associated with a heavy use of pesticides is very widespread in the western part of the Ligurian region (Italy). The formation of micronuclei in peripheral blood lymphocytes is a valuable cytogenetic biomarker in human populations occupationally exposed to genotoxic compounds. In the present study we investigated the micronucleus frequency in peripheral blood lymphocytes of 52 floriculturists and 24 control subjects by use of the cytokinesis-block methodology associated with fluorescence in situ hybridization with a pan-centromeric probe that allowed to distinguish centromere-positive (C+) and centromere-negative (C-) micronuclei. The comparison between floriculturists and controls did not reveal any statistically significant difference in micronucleus frequency, although an increase was observed with increasing pesticide use, number of genotoxic pesticides used and duration of exposure. An increase in C+ as well as in C- micronuclei and in the percentage of C+ micronuclei with respect to the total number of micronuclei was detected in floriculturists, suggesting a higher contribution of C+ micronuclei in the total number scored. The percentage C+ micronuclei was not related to the duration of exposure or to the number of genotoxic pesticides used, but a higher percentage (66.52 % versus 63.78 %) was observed in a subgroup of subjects using benzimidazolic compounds, compared with the floriculturist population exposed to a complex pesticide mixture not including benzimidazolics. These results suggest a potential human hazard associated with the exposure to this class of aneuploidy-inducing carcinogens.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable for glyphosate

Comment:

Well-documented study. MN-test comparable to OECD guidelines, but not equal. No information on exposure concentrations to individual pesticides

Relevance of study:

Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenic non-statistically significant differences possible. No statistically relevant findings reported for glyphosate alone).

Klimisch code:

2

Author(s)	Year	Study title
Cavas, T., Könen S.	2007	Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (<i>Carassius auratus</i>) exposed to a glyphosate formulation using the micronucleus test and the comet assay Mutagenesis 22 263-268 ASB2012-11587

Abstract*

Glyphosate is a widely used broad-spectrum weed control agent. In the present study, an in vivo study on the genotoxic effects of a technical herbicide (Roundup®) containing isopropylamine salt of glyphosate was carried out on freshwater goldfish *Carassius auratus*. The fish were exposed to three doses of glyphosate formulation (5, 10 and 15 ppm). Cyclophosphamide at a single dose of 5 mg/L was used as positive control. Analysis of micronuclei, nuclear abnormalities and DNA damage were performed on peripheral erythrocytes sampled at intervals of 48, 96 and 144 h post treatment. Our results revealed significant dose-dependent increases in the frequencies of micronuclei, nuclear abnormalities as well as DNA strand breaks. Our findings also confirmed that the alkaline comet assay and nuclear deformations in addition to micronucleus test on fish erythrocytes in vivo are useful tools in determining the potential genotoxicity of commercial herbicides.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not Reliable

Comment:

Methodological and reporting deficiencies (e.g. test substance source, no concurrent measurement of toxicity reported, less than 2000 erythrocytes scored per animal and results not reported separately for replicates).

Relevance of study:

Relevant with restrictions (Due to reliability. Discussion confuses glyphosate with glyphosate formulated products.)

Klimisch code:

3

Author(s)	Year	Study title
Guilherme, S. Gaivao, I. Santos, M.A. Pacheco, M.	2010	European eel (<i>Anguilla Anguilla</i>) genotoxic and pro-oxidant responses following short-term exposure to Roundup® - a glyphosate-based herbicide. Mutagenesis Volume: 25 Number: 5 Pages: 523-530 ASB2012-11836

Abstract*

The glyphosate-based herbicide, Roundup®, is among the most used pesticides worldwide. Due to its extensive use, it has been widely detected in aquatic ecosystems representing a potential threat to non-target organisms, including fish. Despite the negative impact of this commercial formulation in fish, as described in literature, the scarcity of studies assessing its genotoxicity and underlying mechanisms is evident. Therefore, as a novel approach, this study evaluated the genotoxic potential of Roundup® to blood cells of the European eel (*Anguilla anguilla*) following short-term (1 and 3 days) exposure to environmentally realistic concentrations (58 and 116 mg/L), addressing also the possible association with oxidative stress. Thus, comet and erythrocytic nuclear abnormalities (ENAs) assays were adopted, as genotoxic end points, reflecting different types of genetic damage. The prooxidant state was assessed through enzymatic (catalase, glutathione-S-transferase, glutathione peroxidase and glutathione reductase) and non-enzymatic (total glutathione content) antioxidants, as well as by lipid peroxidation (LPO) measurements. The Roundup® potential to induce DNA strand breaks for both concentrations was demonstrated by the comet assay. The induction of chromosome breakage and/or segregational abnormalities was also demonstrated through the ENA assay, though only after

3-day exposure to both tested concentrations. In addition, the two genotoxic indicators were positively correlated. Antioxidant defences were unresponsive to Roundup®. LPO levels increased only for the high concentration after the first day of exposure, indicating that oxidative stress caused by this agrochemical in blood was not severe. Overall results suggested that both DNA damaging effects induced by Roundup® are not directly related with an increased pro-oxidant state. Moreover, it was demonstrated that environmentally relevant concentrations of Roundup® can pose a health risk for fish populations.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

No positive controls were included, which significantly detracts from the utility of a non-validated, non-standard test method. Less than the standard of a minimum of three dose levels used, independent coding of slides for scoring and results not reported separately for replicates.

Relevance of study:

Not relevant (Non-standard test system, no positive controls to verify test method/study validity.)

Klimisch code:

3

Author(s)	Year	Study title
Kale, P.G. Petty, B.T. Jr. Walker, S. Ford, J.B. Dehkordi, N. Tarasia, S. Tasie, B.O. Kale, R. Sohni, Y.R.	1995	Mutagenicity Testing of Nine Herbicides and Pesticides Currently Used in Agriculture. Environmental and Molecular Mutagenesis Volume: 25 Pages: 148-153 Z73986, ASB2012-11860

Abstract*

Nine herbicides and pesticides were tested for their mutagenicity using the *Drosophila* sex-linked recessive lethal mutation assay. These are Ambush, Treflan, Blazer, Roundup, 2,4-D Amine, Crossbow, Galecron, Pramitol, and Pondmaster. All of these are in wide use at present. Unlike adult feeding and injection assays, the larvae were allowed to grow in medium with the test chemical, thereby providing long and chronic exposure to the sensitive and dividing diploid cells, i.e., mitotically active spermatogonia and sensitive spermatocytes. All chemicals induced significant numbers of mutations in at least one of the cell types tested. Some of these compounds were found to be negative in earlier studies. An explanation for the difference in results is provided. It is probable that different germ cell stages and treatment regimens are suitable for different types of chemicals. Larval treatment may still be valuable and can complement adult treatment in environmental mutagen testing.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Comparable to 1984 OECD guideline, but with several deficiencies (no positive controls reported and thus study validity not verifiable; wild type male treatment age different than recommended, purity of test substances not reported, tested formulation other ingredients such as surfactants not reported.)

Relevance of study:

Not relevant for glyphosate (Glyphosate not tested; formulation tested)

Klimisch code:

3

Author(s)	Year	Study title
Manas, F. Peralta, L. Raviolo, Garcia Ovando, H. Weyers A. Ugnia, L. Gonzalez Cid, M. Larripa, I. Gorla, N.	2009	Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. Ecotoxicology and Environmental Safety Volume: 72 Pages: 834-837 ASB2012-11891

Abstract*

Formulations containing glyphosate are the most widely used herbicides in the world. AMPA is the major environmental breakdown product of glyphosate. The purpose of this study is to evaluate the in vitro genotoxicity of AMPA using the Comet assay in Hep-2 cells after 4h of incubation and the chromosome aberration (CA) test in human lymphocytes after 48 h of exposition. Potential in vivo genotoxicity was evaluated through the micronucleus test in mice. In the Comet assay, the level of DNA damage in exposed cells at 2.5-7.5 mM showed a significant increase compared with the control group. In human lymphocytes we found statistically significant clastogenic effect AMPA at 1.8 mM compared with the control group. In vivo, the micronucleus test rendered significant statistical increases at 200-400 mg/kg. AMPA was genotoxic in the three performed tests. Very scarce data are available about AMPA potential genotoxicity.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Reporting deficiencies (purity of AMPA not specified, several parameters in the MNT not reported, only 2 dose levels used in both CA and MNT). Exposure route used in the MNT is not relevant for human exposure; methodological deficiencies (see guideline deviations).

Relevance of study:

Not relevant (Due to reliability)

Klimisch code:

3

Author(s)	Year	Study title
Manas, F. Peralta, L. Raviolo, J. Garcia Ovando, H. Weyers, A. Ugnia, L. Gonzalez Cid, M. Larripa, I. Gorla, N.	2009	Genotoxicity of glyphosate assessed by the comet assay and cytogenic tests Environmental Toxicology and Pharmacology Volume: 28 Pages: 37-41 ASB2012-11892

Abstract*

It was evaluated the genotoxicity of glyphosate which up to now has heterogeneous results. The comet assay was performed in Hep-2 cells. The level of DNA damage in the control group (5.42 ± 1.83 arbitrary units) for tail moment (TM) measurements has shown a significant increase ($p < 0.01$) with glyphosate at a range concentration from 3.00 to 7.50mM. In the chromosome aberrations (CA) test in human lymphocytes the herbicide (0.20–6.00mM) showed no significant effects in comparison with the control group. In vivo, the micronucleus test (MNT) was evaluated in mice at three doses rendering statistical significant increases at 400 mg/kg (13.0 ± 3.08 micronucleated erythrocytes/1000 cells, $p < 0.01$). In the present study glyphosate was genotoxic in the comet assay in Hep-2 cells and in the MNT test at 400 mg/kg in mice. Thiobarbituric acid reactive substances (TBARs) levels, superoxide dismutase (SOD) and catalase (CAT) activities were quantified in their organs. The results showed an increase in these enzyme activities.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Guideline deviations and reporting deficiencies. Several parameters in the MNT not reported. Blind scoring reported for the CA but not MNT. Exposure route used in the MNT is not relevant for human exposure. (see guideline deviations). No indication of pH or osmolality control for the comet assay. Results not reported separately for replicates.
Relevance of study:	Not relevant (Due to guideline deviations and reporting deficiencies).
Klimisch code:	3

Author(s)	Year	Study title
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 <i>in Vitro</i> Environmental and Molecular Mutagenesis Volume: 50 Number: 9 Pages: 800-807 ASB2012-11906

Abstract*

In the present study we evaluated the genotoxic and oxidative potential of glyphosate on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure. Testing was done with and without metabolic activation (S9). Ferric-reducing ability of plasma (FRAP), thiobarbituric acid reactive substances (TBARS) and the hOGG1 modified comet assay were used to measure glyphosate's oxidative potential and its impact on DNA. Genotoxicity was evaluated by alkaline comet and analysis of micronuclei and other nuclear instabilities applying centromere probes. The alkaline comet assay showed significantly increased tail length (20.39 μm) and intensity (2.19 %) for 580 $\mu\text{g/mL}$, and increased tail intensity (1.88 %) at 92.8 $\mu\text{g/mL}$, compared to control values of 18.15 μm for tail length and 1.14 % for tail intensity. With S9, tail length was significantly increased for all concentrations tested 3.5, 92.8, and 580 $\mu\text{g/mL}$. Using the hOGG1 comet assay, a significant increase in tail intensity was observed at 2.91 $\mu\text{g/mL}$ with S9 and 580 $\mu\text{g/mL}$ without S9. Without S9, the frequency of micronuclei, nuclear buds and nucleoplasmic bridges slightly increased at concentrations 3.5 $\mu\text{g/mL}$ and higher. The presence of S9 significantly elevated the frequency of nuclear instabilities only for 580 $\mu\text{g/mL}$. FRAP values slightly increased only at 580 $\mu\text{g/mL}$ regardless of metabolic activation, while TBARS values increased significantly. Since for any of the assays applied, no clear dose-dependent effect was observed, it indicates that glyphosate in concentrations relevant to human exposure do not pose significant health risk.

* Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	Non-GLP, non-guideline <i>in vitro</i> study, meeting scientific principles
Relevance of study:	Relevant with restrictions (Assessment of Genotoxicity <i>in vitro</i> at concentrations relevant to human exposure levels; authors state that no clear dose-dependent effect was observed, and results indicate that glyphosate in concentrations relevant to human exposure do not pose significant health risk.
Klimisch code:	2

Author(s)	Year	Study title
Mladinic, M., Perkovic, P., Zeljezic, D.	2009b	Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay Toxicology Letters Volume: 189 Number: 2 Pages: 130-137 ASB2012-11907

Abstract*

Possible clastogenic and aneugenic effects of pesticides on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure were evaluated with and without the use of metabolic activation (S9). To get a better insight into the content of micronuclei (MN) and other chromatin instabilities, lymphocyte preparations were hybridized using pancentromeric DNA probes. Frequency of the MN, nuclear buds (NB) and nucleoplasmic bridges (NPB) in cultures treated with glyphosate slightly increased from 3.5 µg/mL onward. Presence of S9 significantly elevated cytome assay parameters only at 580 µg/mL. No concentration-related increase of centromere (C+) and DAPI signals (DAPI+) was observed for glyphosate treatment. Terbuthylazine treatment showed a dose dependent increase in the number of MN without S9 significant at 0.0008 µg/mL and higher. At concentration lower than 1/16 LD₅₀ occurrence of C + MN was significantly elevated regardless of S9, but not dose related, and in the presence of S9 only NBs containing centromere signals were observed. Carbofuran treatment showed concentration dependent increase in the number of MN. The frequency of C + MN was significant from 0.008 µg/mL onward regardless of S9. Results suggest that lower concentrations of glyphosate have no hazardous effects on DNA, while terbuthylazine and carbofuran revealed a predominant aneugenic potential.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable

Comment: Non-GLP, non-guideline study *in vitro*. Positive and negative control results almost indistinguishable for MN assay without metabolic activation. Negative control NB and NBP results not reported.

Relevance of study: Not relevant (Proposed mechanism of genotoxicity (*in vitro*) is not relevant to human exposure levels. Authors express confidence that estimated maximum human exposure levels correspond to acceptable safety levels based on evaluated *in vitro* endpoints, and that their findings need to be verified *in vivo*.)

Klimisch code: 3

Author(s)	Year	Study title
Paz-Y-Mino, C. Sanchez, M. E. Arevalo, M. Munoz, M. J. Witte, T. De-La-Carrera, G. O. Leone, P. E.	2007	Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. Genetics and Molecular Biology Volume: 30 Number: 2 Pages: 456-460 ASB2012-11992

Abstract*

We analyzed the consequences of aerial spraying with glyphosate 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. The results showed a higher degree of DNA damage in the exposed group (comet length = 35.5 μm) compared to the control group (comet length = 25.94 μm). These results suggest that in the formulation used during aerial spraying glyphosate had a genotoxic effect on the exposed individuals.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable

Comment: Documentation of Comet assay insufficient for assessment.

Relevance of study: Not relevant (Glyphosate formulation was applied at much higher dose rates than recommended for the intended uses in the EU. In addition, the herbicide was combined with the adjuvant (Cosmoflux 411F) that can increase the biological action of the herbicide. This adjuvant will not be used in the EU.)

Klimisch code: 3

Author(s)	Year	Study title
Peluso, M. Munnia, A. Bolognesi, C. Parodi, S.	1998	³² P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. Environmental and Molecular Mutagenesis Volume: 31 Number: 4 Pages: 55-59 TOX1999-318

Abstract*

Roundup is a postemergence herbicide acting on the synthesis of amino acids and other important endogenous chemicals in plants. Roundup is commonly used in agriculture, forestry, and nurseries for the control or destruction of most herbaceous plants. The present study shows that Roundup is able to induce a dose-dependent formation of DNA adducts in the kidneys and liver of mice. The levels of Roundup-related DNA adducts observed in mouse kidneys and liver at the highest dose of herbicide tested (600 mg/kg) were 3.0 +/- 0.1 (SE) and 1.7 +/- 0.1 (SE) adducts/10(8) nucleotides, respectively. The Roundup DNA adducts were not related to the active ingredient, the isopropylammonium salt of glyphosate, but to another, unknown component of the herbicide mixture. Additional experiments are needed to identify the chemical specie(s) of Roundup mixture involved in DNA adduct formation. Findings of this study may help to protect agricultural workers from health hazards and provide a basis for risk assessment.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not Reliable

Comment:

A non-guideline study with confounding results based on testing a surfactant containing formulation.

Reporting deficiencies (statistical methods). Toxic surfactant effects subsequently verified in Heydens et al. (2008, ASB2012-11845) reporting the same study type with a glyphosate formulated product and an appropriate control; formulation blank without glyphosate.

Relevance of study:

Not relevant (i.p. administration of high doses of a surfactant containing formulation a relevant exposure scenario for human risk assessments. In addition, the DNA adducts observed were not related to the active ingredient (isopropylammonium salt of glyphosate), but to another, unknown component of the herbicide mixture.)

Klimisch code:

3

Author(s)	Year	Study title
Poletta, G.L. Larriera, A. Kleinsorge, E. Mudry, M.D.	2009	Genotoxicity of the herbicide formulation Roundup® (glyphosate) in broad-snouted caiman (<i>Caiman latirostris</i>) evidenced by the Comet assay and Micronucleus test Mutation Research Volume: 672 Number: 2 Pages: 95-102 ASB2012-12002

Abstract*

The genotoxicity of pesticides is an issue of worldwide concern. The present study was undertaken to evaluate the genotoxic potential of a widely used herbicide formulation, Roundup® (glyphosate), in erythrocytes of broad-snouted caiman (*Caiman latirostris*) after in ovo exposure. Caiman embryos were exposed at early embryonic stage to different sub-lethal concentrations of Roundup® (50, 100, 200, 300, 400, 500, 750, 1000, 1250 and 1750 µg/egg). At time of hatching, blood samples were obtained from each animal and two short-term tests, the Comet assay and the Micronucleus (MN) test, were performed on erythrocytes to assess DNA damage. A significant increase in DNA damage was observed at a concentration of 500 µg/egg or higher, compared to untreated control animals ($p < 0.05$). Results from both the Comet assay and the MN test revealed a concentration-dependent effect. This study demonstrated adverse effects of Roundup® on DNA of *C. latirostris* and confirmed that the Comet assay and the MN test applied on caiman erythrocytes are useful tools in determining potential genotoxicity of pesticides. The identification of sentinel species as well as sensitive biomarkers among the natural biota is imperative to thoroughly evaluate genetic damage, which has significant consequences for short- and long-term survival of the natural species.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Non-GLP studies in a unique test model. Micronucleus assay followed guideline, Comet assay similar to guideline.

Test methods have been modified to be applied caiman species. Methodological deficiencies: housing and feeding conditions of parents not specified; sex not distinguished, stability and homogeneity assessment of test substance preparations not reported. Results not reported separately for replicate individual animals.

Relevance of study:

Not relevant. Highly artificial *in ovo* exposure scenario not relevant to real world environmental exposures. Caiman eggs are covered and not exposed to the surface. Any glyphosate in a potential herbicide overspray would sorb to sediment and organic matter. and not transport to the egg surface.

Klimisch code:

3

Author(s)	Year	Study title
Rodrigues, H.G. Penha-Silva, N. Ferreira Pereira de Araujo, M. Nishijo, H. Aversi-Ferreira, T.A.	2011	Effects of Roundup [®] Pesticide on the Stability of Human Erythrocyte Membranes and Micronuclei Frequency in Bone Marrow Cells of Swiss Mice The Open Biology Journal, Volume: 4 Pages: 54-59 ASB2012-12010

Abstract*

Pesticides can affect the health of living organisms through different mechanisms such as membrane denaturation. The evaluation of the deleterious effects of chemical agents on biological membranes can be performed through the analysis of the stability of erythrocytes against a concentration gradient of certain chemical agent in physiologic saline solution. This work analyzed the effect of the herbicide Roundup[®] on the membrane of human erythrocytes in blood samples collected with EDTA or heparin as anticoagulant agent. The results were analyzed through spectrophotometry at 540 nm and light microscopy. There was an agreement between spectrophotometric and morphologic analyses. At the concentration limit recommended for agricultural purposes, Roundup[®] promoted 100 % of hemolysis. The D₅₀Roundup[®] values obtained for human blood samples collected with EDTA were not significantly different from those obtained for samples collected with heparin. However, the lysis curves presented lower absorbance values at 540 nm in the presence of blood collected with EDTA in relation to that collected with heparin, probably due to haemoglobin precipitation with EDTA. This work also analyzed the effects of three different Roundup[®] doses (0.148, 0.754 and 1.28 mg/kg) on the micronuclei frequency in bone marrow cells of Swiss mice in relation to a positive control of cyclophosphamide (250 mg/kg). The two highest Roundup[®] doses showed the same genotoxicity level as the positive control.

* Quoted from article

Klimisch evaluation

1 Reliability of study:

Not reliable. Determination of the stability of human erythrocytes: Results are not surprising because surfactants are known to compromise cell membrane integrity. Doses not reflective of physiological concentrations of either glyphosate or surfactant.

Micronucleus test *in vivo*: Irrelevant route of exposure for surfactant containing formulated products. Results confounded by presence of surfactant toxicity; refer to Heydens et al. (2008, ASB2012-11845)

Comment:

Non-guideline, non-GLP studies

Determination of the stability of human erythrocytes
Results attributable to surfactant induced cytotoxicity

Micronucleus test *in vivo*

Major reporting deficiencies (no information on number of cells evaluated, only graphical documentation of results, no information on absolute MN frequencies).

Relevance of study:

Not relevant (Test material containing surfactant is not appropriately evaluated in either model).

Klimisch code:

3

Author(s)	Year	Study title
Vigfusson, N.V. Vyse, E.R.	1980	The effect of the pesticides Dexon, Captan and Roundup on sister chromatid exchanges in human lymphocytes in vitro. Mutation Research Volume: 79 Pages: 53-57 TOX9700576, ASB2012-12044

Abstract*

Three pesticides at varying concentration were tested for the induction of SCE [sister chromatid exchanges] in human lymphocytes in vitro. The fungicide, Dexon, sodium (4-(dimethylamino)phenyl)diazene sulfonate, caused the greatest increase in SCE frequency and the response was dose related. The herbicide, Roundup, isopropylamide salt of N-(phosphonomethyl)glycine, had the least effect on SCE requiring the use of much higher concentrations to produce an effect. Limited results were obtained with the fungicide Captan, cis-N-((trichloromethylthio)-4-cyclo-hexene-1, 2-dicarboximide, because of toxic levels of the fungicide or solvent used.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Test material was a formulated product containing surfactant. Authors acknowledge cytotoxicity was a confounding factor for data interpretation; since the time of this study, around 1980, surfactant effects on *in vitro* test systems have been well documented. Only very minor changes in SCE were reported, with a limited data set of two donors and a lack of dose-response. Statistical analysis was not feasible with this very limited data set.

Relevance of study:

Not relevant (Limited data set, internally consistent findings, no statistics conducted and no dose-response)

Klimisch code:

3

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)

Introduction into this chapter by the RMS

The chronic toxicity/carcinogenicity part is mainly based on the extensive descriptions of the available valid studies which were provided by the GTF in its dossier. It was noted that a different approach was taken in the dossier with regard to the studies in rats and those in mice. In the section compiling the rat studies, all of them were reported in detail, including the four long-term studies that had been reviewed during previous EU evaluation. In the section on studies on the mouse, only the new studies are described whereas for those already known reference to the old DAR (DAR, 1998, ASB2010-10302) was made.

For higher efficiency of the review and for the sake of transparency, the descriptions of methods and study results in the GTF dossier were virtually not amended and even the conclusions were kept as provided. However, each study that is described in detail was commented by RMS. These remarks on bottom of each study description are clearly distinguished from the original submission by a caption and are always written in italics. In addition, redundant parts (in particular the so-called "executive summaries") have been deleted and the structure of the original submission was significantly changed to make it more transparent and comprehensible.

With regard to the "old" studies in mice that were not reported in the GTF dossier once more, at least re-evaluation for quality and reliability was performed by the RMS and the NOAELs/LOAELs were checked.

A paragraph on testing of formulations for long-term effects in rats has been included.

The overall assessment of chronic toxicity/carcinogenicity of glyphosate by the RMS is provided in Vol. 1 (2.6.5).

In chapter B.6.5.3 publications on glyphosate and carcinogenicity are presented. These publications include a number of epidemiology studies which are focused on pesticide exposure and associated health outcomes.

B.6.5.1 Long-term toxicity and carcinogenicity in the rat

B.6.5.1.1 New studies in rats

1st study: [REDACTED] **1996**

Reference: IIA, 5.5.1/01

Report: [REDACTED] (1996)

Glyphosate Acid: One Year Dietary Toxicity Study in Rats

[REDACTED],
Cheshire, UK

Data owner: Syngenta

Study No.: [REDACTED]/P/5143

Date: 1996-10-02

not published TOX2000-1998

Guidelines: OECD 452, US EPA 83-1

Deviations: Several organ weights not determined

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 1995-04-03 - 1996-06-03

Materials and methods

Test material:

Identification: Glyphosate acid

Description: White solid

Lot/Batch #: P24

Purity: 95.6 %

Stability of test compound: At least 1 year when stored at RT.

Vehicle and/
or positive control: Diet

Test animals:

Species: Rat

Strain: Wistar (Alpk:AP_rSD)

Source:

Age: 22-24 days (on delivery)

Sex: Males and females

Weight at dosing: Males: 150.5 – 151.5 g (mean values); females: 126.7 – 133.3 g (mean values)

Acclimation period: At least 10 days

Diet/Food: CT1 diet (Special Diet services Ltd., Essex, UK), *ad libitum*

Water: Mains drinking water, *ad libitum*

Housing: Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage.

Environmental conditions: Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: at least 15/hour
12 hours light/dark cycle

In life dates: 1995-04-03 to 1996-06-03

Animal assignment and treatment:

In a chronic toxicity study groups of 24 Wistar-derived rats per sex received daily dietary doses of 0, 2,000, 8,000 and 20,000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females).

Test diets were prepared in either 30 or 60 kg batches by mixing the appropriate amount of the test substance with the basal diet. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20000 ppm.

Observations

Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study.

The eyes of the control and high dose group were additionally examined one week to termination.

Body weight

Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 14 and every two weeks thereafter until termination.

Food consumption and compound intake

Food consumption was recorded once weekly for each cage group from Week 1 to Week 13, once in Week 16 and every fourth week thereafter.

Haematology and clinical chemistry

Blood was collected from 12 animals per sex and group at Week 14, 27 and at termination (Week 53). The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ -glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis

Individual urine samples were collected from the same animals as those used for haematology analyses (except for Week 52) at Week 13, 26 and 52. The following parameters were determined: Volume, colour, appearance, specific gravity, pH, glucose, ketones, protein, urobilinogen and blood.

Sacrifice and pathology

Necropsy was conducted on all animals except for Rats 38 and 149-152, which were killed during Week 6/7 due to a sexing error. The following organ weights were determined from all animals surviving to scheduled termination: Adrenals, brain, epididymides, kidneys, liver and testes.

Tissue samples were taken from the following organs: Adrenals, aorta, bone & bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eye, gross lesions, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, nasopharyngeal cavity, sciatic nerve, oesophagus, oral cavity, ovary, pancreas, pituitary, prostate, rectum, salivary glands, seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and voluntary muscle.

Statistics

All data were evaluated using analysis of variance and covariance for each specified parameter using the GLM procedure in SAS (1989). Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis. All statistical tests were two sided.

Results and discussion

Analysis of dose formulations

The mean achieved concentrations of glyphosate acid in each dietary preparation were within 8 % of the nominal concentration and the overall mean concentrations were within 4 % of nominal.

The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20000 ppm was satisfactory; percentage deviations were within 7 % of the overall mean.

The stability tests determined at 2000 and 20000 ppm showed that the test substance is stable for at least 61 days when stored at room temperature.

Mortality

There were no treatment-related death.

Clinical observations

There was a small increase in the number of animals in the 20000 ppm group which had urinary staining (wet or dry). All other clinical observations were of a type and incidence expected for rats of this strain.

Body weight

Body weights of rats receiving 20000 ppm glyphosate acid were lower than those of controls throughout the study. Bodyweights in the intermediate group were slightly reduced throughout the study. The difference from control was not statistically significant in males and was statistically significant in females only from Week 46. As the pattern of the effect was similar to that of the high dose rats for both sexes this minor difference in bodyweight is considered to be related to administration of glyphosate acid.

There was no effect on bodyweight in rats receiving 2000 ppm glyphosate acid.

Food consumption and compound intake

Food consumption was generally lower in rats receiving 20000 ppm than in controls. The difference was most marked at the start of the study. Food consumption was generally slightly lower than controls in rats receiving 8000 ppm glyphosate acid. There was no effect on food consumption in rats receiving 2000 ppm.

The group mean achieved doses are summarised below.

Table B.6.5-1: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	2000	141	167
3 (mid)	8000	560	671
4 (high)	20000	1409	1664

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.

Ophthalmoscopy

There were no treatment-related effects observed.

Haematology and clinical chemistry

Haematology

A number of statistically significant differences from control were identified but there was no evidence of a relationship to dose and the differences were small and not seen consistently at all the time points and therefore were considered to be unrelated to glyphosate acid administration.

Clinical chemistry

Plasma cholesterol and plasma triglycerides were marginally reduced in males receiving 20000 or 8000 ppm at Weeks 14 and 27.

Moreover, there was a treatment- and dose-related increase in plasma ALP activity throughout the study. For rats receiving 2000 ppm glyphosate acid the increase was marginal and was statistically significant only for females at Week 14. The increase in the activity of plasma ALP in animals at all dose levels was compound-related but as there was no accompanying pathological change in either the liver or bone this is considered not to be of toxicological significance.

All other differences from control were small and/or were not dose-related and are considered to be incidental to administration of glyphosate acid.

Table B.6.5-2: Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Alkaline Phosphatase (IU/L)								
Week 14	248	161	281	201*	342**	227**	429**	292**
Week 27	221	135	250	171	306**	200**	412**	254**
Week 53	232	87	258	100	291**	114	379**	160**

* p < 0.05; ** p < 0.01

Urinalysis

There were no consistent treatment- and dose-related effects seen in the any urinary parameters.

Necropsy

Gross pathology

There were no treatment-related macroscopic effects.

Organ weights

There were no treatment- and dose-related effects on organ weights when corrected for bodyweight.

Histopathology

An increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland were seen in both sexes receiving 20000 ppm glyphosate acid. This change was considered to be related to treatment and consequently the salivary glands of the 8000 ppm dose group were examined. The examples of focal parotid basophilia seen at this dose were all of minimal severity and the incidence was comparable to that in the control group.

All other observed differences in the incidence of findings are considered to be unrelated to the treatment with glyphosate acid in view of the spontaneous incidence in this strain. No treatment-related neoplasms were found.

Conclusion by the Notifiers

Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 12 month is 8000 ppm (corresponding to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females).

RMS comment:

The study is considered acceptable. In addition to the effects described, food utilization was less efficient at the top dose level. A certain increase in basophilia of acinar cells of the parotid salivary gland was still noted in mid dose females although the incidence in males was indeed comparable to the control group (see additional Table B.6.5-3).

Table B.6.5-3: Incidence of focal basophilia in parotid salivary gland of rats treated with glyphosate acid for one year

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Focal basophilia								
Minimal	2	2	0	0	3	6	10	8
Slight	0	0	0	0	0	0	3	5
Moderate	0	0	0	0	0	0	0	2

Based on this histopathological alteration, the lower body weight even at the mid dose level towards the end of the study, a lower food consumption and higher AP activity at the two upper dose levels, the NOAEL in this chronic study is rather seen at 2000 ppm (equal to a mean daily intake of 141 or 167 mg/kg bw in male or female rats) instead of the next higher dose as suggested by GTF. There was no evidence of carcinogenicity but duration of treatment was too short for a definitive conclusion.

2nd study: [REDACTED] **1997**

Reference: IIA, 5.5.2/02

Report: [REDACTED] (1997)

HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats

[REDACTED]

Data owner: Arysta Life Sciences

Study No.: IET 94-0150

Date: 1997-07-01

not published,

ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487

Guidelines: OECD 453 (1981), JMAFF 59 NohSan 3850 (1984), US-EPA (1989)

Deviations: None

GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 1994-12-19 - 1996-12-25

Materials and methods

Test material:

Identification: Glyphosate technical, Code: HR-001

Description: White crystal

Lot/Batch #: T-941209; T-950308

Purity: 97.56 %; 94.61 %

Stability of test compound: No data given the report.

Vehicle and/
or positive control: Diet

Test animals:

Species: Rat

Strain: Sprague-Dawley (Crj:CD)

Source:

Age: 5 weeks (males), 6 weeks (females)

Sex: Males and females

Weight at dosing: 65 – 85 g

Acclimation period: At least one week

Diet/Food: MF Mash (Oriental Yeast Co., Ltd, Japan), *ad libitum*

Water: Well water treated with sand and charcoal filter, HCl and UV rays, *ad libitum*

Housing: In groups of ten animals of the same sex in wire-mesh stainless steel cages during the acclimatisation period. During the study males were housed in groups of 5 per cage until week 72, in groups of ≤ 3 until week 78 and individually thereafter. Females were housed in groups of five until week 78, and individually thereafter.

Environmental conditions: Temperature: 24 ± 2 °C
 Humidity: 55 ± 15 %
 Air changes: 15/hour
 12 hours light/dark cycle

Study design and methods:

In life dates: 1994-12-19 to 1996-12-25

Animal assignment and treatment:

In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats/sex/group received daily dietary doses of 0, 3000, 10000 and 30000 ppm (equivalent to mean achieved dose levels of 0, 104, 354 and 1127 mg/kg bw/day in males and 0, 115, 393 and 1247 mg/kg bw/day in females) HR-001. In addition, 30 rats/sex/group were included for interim sacrifices at 26, 52 and 78 weeks.

Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet. This pre-mix was then added to a larger amount of basal diet and blended by a blending machine.

The stability of the test substance in food was previously determined in a 4-week dose-range finding study in mice. Homogeneity analyses were performed on samples of each dose level of the first diet preparation. Analyses for achieved concentrations were done for each dose level in monthly intervals.

Observations

Rats of all groups were examined for toxic signs and pre-terminal deaths once a day. In addition a detailed veterinary examination was made at least once per week. Ophthalmic examination was done at the start of the study and at termination.

Body weight

Individual body weights were recorded at weekly intervals until the end of Week 13 and every 4 weeks thereafter and before necropsy, except for dead or moribund satellite animals, which were discarded without body weight determination.

Food consumption and compound intake

Food consumption was measured for a period of three consecutive days weekly from Week 1 to 13 and every four weeks from Week 16 to 104. Mean individual food consumption, group mean food consumption and group compound intake were calculated.

Haematology and clinical chemistry

Blood samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in week 104. Before sampling animals were fasted overnight. The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, alkaline phosphatase, glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), γ -glutamyl-transpeptidase, creatine phosphokinase, creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis

Individual urine samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in Week 104. The following measurements were made: density, volume, appearance, pH, protein, glucose, occult blood, ketones, urobilinogen, sediments.

Sacrifice and pathology

Necropsy and histopathological examinations were carried out on all tissues collected at interim and terminal sacrifice. The following organ weights were determined from all animals: adrenals, brain, caecum, kidneys, liver and testis.

Tissue samples were taken from the following organs: adrenals, aorta, bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons and medulla oblongata), caecum, colon, duodenum, epididymides, eyes, gross lesions, Harderian glands, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands (submaxillary and

sublingual), sciatic nerve, seminal vesicles and coagulating glands, skeletal muscle, skin (females only), spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (horns and cervix) and vagina.

Statistics

Statistical significance of the difference between the control group and the treated groups was estimated at 5 % and 1 % levels of probability.

The data of body weight (main group only), food consumption, urine specific gravity, urine volume, haematological parameters, blood biochemical parameters, and organ weights were evaluated by Bartlett's test for equality of variance. When group variances were homogeneous, a parametric analysis of variance of a one-way layout type was conducted to determine if any statistical differences existed among groups. When the analysis of variance was significant, Dunnett's (when sample size of each group was equal) or Scheffé's (when sample size of each group was different) multiple comparison test was applied to evaluate differences between the treated and the control groups. When the group variances were heterogeneous, the data were analyzed by Kruskal-Wallis, non-parametric analysis of variance. When significant, Dunnett type (when sample size of each group was equal) or Scheffé type (when sample size of each group was different) mean rank sum test was applied to determine if any significant differences existed between the treated and the control groups. The data of urinalysis except for specific gravity and urine volume were assessed by Mann-Whitney's U test.

Mortality was analyzed by Life table analysis.

The data of clinical sign (main group only), ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability test.

Results and discussion

Analysis of dose formulations

The coefficient of variation for the homogeneity of the test substance for each dose level was 2.2% and less. Hence, the results indicated a good homogeneity.

Analyses for concentrations showed that the diet preparations achieved 97 - 98% of the target concentration. Thus, the concentrations of the test substance in the test diets were within acceptable limits.

Mortality

In the high dose group neither sex showed an increase in mortality, although mortality in males was lower than the control during the last half of the treatment period with statistical significance in most of the weeks. In all other groups mortality was comparable to control. The final mortality is given in Table B.6.5-4:

Table B.6.5-4: Final mortality at termination of treatment (%)

	Dose group (ppm)*			
Sex	0	3000	10000	30000
Male	32/50 (64)	30/50 (60)	32/50 (64)	21/50 (42)
Female	35/50 (70)	31/50 (62)	34/50 (68)	36/50 (72)

* number of mortalities / total number of rats/group (% mortality)

Clinical observations

In the high dose group, significant increases in incidence of bradypnea, mass, and soiled fur were observed in males when compared to the control. Analysis of location of each mass showed that the ones in the tail were present in 27 males, which was apparently high in

incidence compared to 11 of the control. The incidences of mass in other locations were comparable to the control. With respect to soiled fur, the sign was located at the external genital or perianal region. Males in this group also showed significant decreases in incidence of tactile hair loss, wound, and hair loss. In females, a significant increase in incidence of wetted fur was observed; the sign was mainly seen in the external genital region. Besides the signs mentioned above, loose stool was observed in all cages of this group from Week 24 in males and Week 23 in females until the end of the treatment. Animals showing loose stool could not be identified because of group housing, therefore the sign is only described here in the text but not included in Table B.6.5-5.

In the mid dose group, the incidence of tactile hair loss was significantly decreased in males and significantly increased in females when compared to the respective control.

In the low dose group, significant increases in incidence of decreased spontaneous motor activity, bradypnea, and soiled fur and a significant decrease in incidence of tactile hair loss were observed in males. Analysis of location of the soiled fur demonstrated predominant occurrences of the sign in the external genital region and foreleg. Females in this group showed significant increases in incidence of ptosis and tactile hair loss.

Table B.6.5-5: Statistically significant changes in clinical signs

	Dose group (ppm)							
	0		3000		10000		30000	
Parameters	♂	♀	♂	♀	♂	♀	♂	♀
Decreased spontaneous motor activity	9	23	19*	22	9	20	13	26
Bradypnea	3	7	10*	14	4	6	11*	12
Ptosis	7	4	6	12*	4	6	6	6
Tactile hair loss	5	1	0*	17**	0*	9**	0*	4
Integument								
Wound	7	2	4	2	6	2	0**	1
Mass	22	37	26	36	21	38	37**	43
Hair loss	12	16	7	13	15	21	3*	25
Soiled fur	10	16	20*	17	12	11	21*	18
Wetted fur	9	5	7	5	7	5	16	15*

* $p < 0.05$; ** $p < 0.01$

Body weight

In the high dose group, body weights were lower than the control throughout the treatment period; significant decreases in their body weights were observed during Weeks 1 to 80 in males and at Week 7 and during Weeks 9 to 60 in females. The final group mean body weights of males and females at termination of the treatment period were both 93% of the respective control.

In the mid dose group, males showed a decreased body weight gain during the first few weeks of treatment with a statistically significant difference from the control at Week 6. Their retarded growth persisted throughout the treatment period, and the final group mean body weight at termination of treatment was 95 % of the control. Body weight change in females was comparable to the control throughout the treatment period.

In the low dose group, body weights of both sexes were comparable to the control except for a significant increase in females at Week 16.

Food consumption and compound intake

In the high dose group, consistent with the decreasing body weight or decreasing body weight trends, food consumption showed a decreasing trend in males during the first few weeks.

In the other groups, food consumption in males and females was comparable to the respective control.

The group mean achieved doses are summarised below.

Table B.6.5-6: Group mean achieved dose levels in the main groups

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	3000	104	115
3 (mid)	10000	354	393
4 (high)	30000	1127	1247

The results show a higher test material intake for females when compared to males for each dose level.

Ophthalmologic examinations: No abnormalities were observed.

Haematology and clinical chemistry: Haematological and blood biochemical analyses did not demonstrate apparent toxicity of the test substance in either sex or group.

Statistically significant changes in haematology and blood chemistry are displayed in Table B.6.5-7 and Table B.6.5-8.

Table B.6.5-7: Statistically significant changes in haematology

	Dose group (ppm)					
	3000		10000		30000	
Parameters	♂	♀	♂	♀	♂	♀
Haematocrit	108 ^a	99	117	84	131*	96
Platelet count	91	105	88	115	66**	104

^a Figures represent values in the treated groups when the corresponding control is 100.

* p < 0.05; ** p < 0.01

Table B.6.5-8: Statistically significant changes in blood chemistry

		Dose group (ppm)					
		3000		10000		30000	
Parameters		♂	♀	♂	♀	♂	♀
Alkaline phosphatase	Week 52	129 ^a	127	145	118	136	214**
	Week 78	185*	303	154	106	171	116
Glutamic pyruvic transaminase	Week 52	94	91	148	75	67*	66
Creatinine	Week 26	102	95	99	91*	97	89**
Total protein	Week 52	100	101	100	96*	99	96
Albumin	Week 26	100	92*	100	99	103	95
Globulin	Week 26	98	102	95	95	95	95*
	Week 52	103	101	101	93*	100	99
Glucose	Week 26	101	104	107	99	97	87**
Total bilirubin	Week 26	100	80**	94	96	106	88
Chloride	Week 104	97*	100	97*	100	98	101

^a Figures represent values in the treated groups when the corresponding control is 100.

* p < 0.05; ** p < 0.01

Urine analysis

Urinalysis did not demonstrate apparent toxicity of the test substance in either sex or group. Statistically significant changes in urinalysis parameters are displayed in Table B.6.5-9.

Table B.6.5-9: Statistically significant changes in urinalysis

		Dose group (ppm)					
		3000		10000		30000	
Parameters		♂	♀	♂	♀	♂	♀
pH	Week 26			↓*		↓**	↓
	Week 52			↓*		↓**	↓**
	Week 78			↓		↓*	↓
	Week 104			↓		↓**	↓
Protein						↓*	
Volume		↑*					
Appearance			Dark*		Dark*		Dark*

* $p < 0.05$; ** $p < 0.01$

Metabolism of glyphosate after absorption from the intestine is minimal. Thus, most of the glyphosate is excreted via urine as the unchanged parent compound. In the urine glyphosate dissociates into the free acid, which can lead to a reduction of the urinary pH. Therefore, the reduced urinary pH might be of no toxicological significance.

Necropsy

In the high dose group significant increases in incidence of distension of the caecum were observed in both sexes, accompanied by soiled fur in the perianal region in males. Moreover, significant increases in absolute and relative weights of the caecum in both sexes in the high and mid dose group were seen, but not associated with histopathological abnormalities.

The incidences of thickened areas in the skin of the tail, corresponding to the tail mass in the clinical observations, were significantly increased in the mid and high dose group. The lesion was histopathologically diagnosed as follicular hyperkeratosis and/or folliculitis/follicular abscess. An increased incidence of hair loss was also observed in high-dosed females, but it lacked corresponding histopathological changes.

All changes regarding neoplastic lesions were not statistically significant.

From this, it is concluded that the test compound at the doses tested does not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.

Conclusion by the Notifiers

Based on the slight body weight effects, and necropsy findings without correlating histopathology at the mid-dose the NOAEL in rats after chronic exposure to HR-001 for 24 month is 3000 ppm (corresponding to 104 mg/kg bw/day for males and 115 mg/kg bw/day for females). It is concluded that HR-001 is not carcinogenic in rats.

RMS comment:

The study is acceptable. We agree with the evaluation by the notifier and support the NOAEL of 104 mg/kg bw/day.

3d study: [REDACTED] **1997**

Reference: IIA, 5.5.2/07
Report: [REDACTED] (1997)
 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat
 [REDACTED]
 Study No.: 1231
 Date: 1997-02-15
 not published, ASB2012-11489
Guidelines: OECD 453 (1981)
Deviations: Organ weights were not determined for all animals; weights of heart, spleen and (para)thyroids are missing
GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 1994-06-09 - 1996-06-12

Materials and methods

Test material:
 Identification: Glyphosate technical
 Description: No data given in the report.
 Lot/Batch #: No data given in the report.
 Purity: No data given in the report.
 Stability of test compound: No data given in the report.
 Vehicle and/
 or positive control: Diet
 Test animals:
 Species: Rat
 Strain: Sprague-Dawley
 Source: Indian Institute of Toxicology (I.I.T.), Pune, India
 Age: Approx. 6 weeks
 Sex: Males and females
 Weight at dosing: Males: 70.0 – 93.2 g, females: 70.0 – 90.6 g
 Acclimation period: One week
 Diet/Food: Powdered rat feed (Lipton India Ltd, India), *ad libitum*
 Water: Filtered pure water, *ad libitum*
 Housing: Initially in groups of five in polypropylene cages, in groups of three from Week 24 to 52 and in groups of two from Week 53 to termination.
 Environmental conditions: Temperature: 22 - 25 °C
 Humidity: 50 - 70 %
 Air changes: 10 - 15/hour
 12 hours light/dark cycle

In life dates: 1994-06-09 to 1996-06-12

Animal assignment and treatment:

In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague Dawley rats per sex received daily dietary doses of 0, 3000, 15000 and 25000 ppm (equivalent to mean achieved dose levels of 0, 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0, 0.21, 1.06 and 1.74 g/kg bw/day (females)) Glyphosate technical for two years. In addition, for the control and each dose group 20 rats per sex were included for interim sacrifice in Week 52 to study non-neoplastic histopathological changes (chronic toxicity study). Selected dose levels were the same except for the highest dose which was 30000 ppm. Here the dietary doses correspond to 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively

Test diets were prepared weekly by mixing appropriate amounts of the test substance with the basal diet. The stability and homogeneity of the test substance in food was determined in-house stability study at all dose levels before the start of dosing. Analyses for achieved concentrations were performed monthly during the study period.

Clinical observations

Rats were examined for toxic signs once and pre-terminal deaths twice a day. Ophthalmic examination was done at the start of the study, at interim sacrifice and at termination in the control and high dose group

Body weight

Individual body weights were recorded on Day 0, at weekly intervals thereafter until the end of Week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake

Food consumption was recorded once weekly for each group from Week 1 to Week 13 and subsequently in Week 25, 38, 51, 65, 78, 92 and 104.

Haematology and clinical chemistry

Haematology

Individual blood samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Haemoglobin, erythrocyte count, PCV, thrombocytes, total leukocyte count and differential leukocyte count.

Blood chemistry

Individual plasma samples were collected from 10 rats/sex/group of the main groups at 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Total serum proteins, albumin, ALT, AST, GGTP, SAP, blood urea nitrogen and blood glucose.

Urinalysis

Individual urine samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. The following measurements were made: Specific gravity, volume, appearance, pH, protein, glucose, occult blood, ketones, microscopy of sediments.

Sacrifice and pathology

Necropsy was performed on all animals at scheduled termination.

The following organ weights were determined from 10 rats per sex per main group and on all animals of the satellite groups: adrenals, brain, gonads, kidneys and liver.

Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups.

Tissue samples were taken from the following organs of all animals: adrenals, aorta, body cavities, brain, caecum, colon, duodenum, epididymis, eyes (both), femur, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric and mandibular), mammary gland, oesophagus, ovaries, pancreas, pituitary, preputial gland, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.

Statistics

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier. Animals withdrawn from study during the interval (those taken for moribund sacrifice) are taken into consideration by giving enough weightage.

The incidence of neoplasms was analysed by Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.

In addition to these tests the Fisher exact test for pairwise comparisons and the Cochran Armitage linear trend test for dose response trends were carried out. All reported P-values for the tumour incidence analysis are one-sided.

The biochemical, haematological and organ weight data was analyzed for significance using Student 't' test or Cochran 't' test.

Results and discussion

Analysis of dose formulations

Analyses for concentrations showed that the diet preparations recovered 86.1 - 98.3 % of the target concentration. Thus, the concentrations of the test substance in the test diets were within acceptable limits.

Analyses for homogeneity recovered 87.5 - 90.0 % for 3000 ppm, 91.7 - 93.0 % for 15000 ppm, 94.3 - 95.1 % for 25000 ppm and 91.8 - 92.6 % for 30000 ppm. Hence, the results indicated a good homogeneity.

Moreover, stability analyses showed that recovery one month after diet preparation ranged between 87.5 and 95.0 %.

Mortality

No treatment-related clinical signs or deaths were observed in the satellite groups, e.g. the chronic toxicity study.

In the carcinogenicity study, e.g. after 104 weeks, male animals of the high dose group exhibited slight but statistically insignificant higher mortalities.

The numbers of pre-terminal deaths in the main group are displayed in Table B.6.5-10.

Table B.6.5-10: Cumulated mortalities after 104-week dietary exposure to Glyphosate technical*

	Dose group (ppm)			
Sex	0	3000	15000	25000
Male	16/50	17/50 (2)	18/50 (4)	23/50 (14)

Female	19/50	20/50 (2)	20/50 (2)	25/50 (12)
--------	-------	-----------	-----------	------------

* Values in parentheses indicate increases in mortality compared to control in percent.

Clinical observations

No significant toxic signs were observed in treated or control groups.

Body weight

Significantly reduced body weight gain that lasted throughout study until Week 104 was observed in males receiving the highest dose. In all other groups body weight gain was comparable to the control at termination.

Food consumption and compound intake

There were no treatment-related effects on food consumption for either sex or group noted during the study.

The results show a higher test material intake for females when compared to males for each dose level. The mean intake in the chronic toxicity study for each dose group is 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively.

The mean intake in the carcinogenicity study for each dose group is 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0.21, 1.06 and 1.74 g/kg bw/day (females) for 3000, 15000 and 25000 ppm, respectively.

The group mean achieved doses are summarised below.

Table B.6.5-11: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)*	
		Males	Females
		Chronic toxicity study (52 weeks)	
low	3000	0.18	0.24
mid	15000	0.92	1.13
high	30000	1.92	2.54
		Carcinogenicity study (104 weeks)	
low	3000	0.15	0.21
mid	15000	0.78	1.06
high	25000	1.29	1.74

* Calculations were done with values from Week 13 (chronic) and Week 25 (carcinogenicity)

Ophthalmological examinations

Ophthalmological examinations revealed no abnormalities.

Laboratory investigation

Haematological examination did not reveal any abnormalities attributable to the treatment. Regarding the clinical chemical investigations, a significant increase in the alkaline phosphatase level was only seen in the high dose of the carcinogenicity study at study termination (see Table B.6.5-12).

Other significant changes observed in haematological, and biochemical parameters were within the range of the historical control data and hence appear to be of no biological significance.

Table B.6.5-12: Statistically significant changes in blood chemistry

	Dose group (ppm)							
	0		3000		15000		25000	
Parameters	♂	♀	♂	♀	♂	♀	♂	♀
Alkaline phosphatase								
Month 6	25.58	24.96	24.97	25.25	24.85	25.2	23.07	25.11
Month 12	25.64	19.04	25.96	25.35*	27.64	28.3*	22.88	22.88*
Month 18	27.7	24.47	25.94	28.42	28.73	27.71	26.68	25.28
Month 24	26.04	24.87	26.75	26.95*	28.42*	25.75	47.71*	53.86*

* p < 0.05

Urinalysis

Urinalysis did not reveal any abnormalities attributable to the treatment.

Necropsy**Gross pathology**

There were no treatment-related macroscopic findings observed during the study period.

Organ weights

Significant and dose-dependent effects in the chronic toxicity study were found in both sexes of the high-dose group. In males, weights of kidneys, brain and testes were increased. In females, in addition to kidneys and brain, the liver weight was increased as well.

In the carcinogenicity study which lasted 52 weeks longer, significant and dose-dependent effects in males consisted of increased weight of brain and testes in the mid and high dose group. Effects on the kidneys were not observed, perhaps due to the lower dose level in the highest group compared to the chronic toxicity study, e.g. 25000 ppm to 30000 ppm, respectively.

In females, significant and dose-dependent effects after 24 months occurred only in kidneys. Like for male animals, this increase could be due to the different high dose levels.

Histopathology

Histopathological changes were found at all dose levels including control, hence it is concluded that these are no treatment-related effects.

Neoplastic changes

There were no treatment-related neoplasms observed.

Conclusion by the Notifiers

Based on the mild toxic effects on body weight gain and the increased organ weights without histopathological changes the NOAEL in rats after chronic exposure to Glyphosate technical for 24 month is 25,000 ppm (corresponding to 1290 mg/kg bw/day for males and 1740 mg/kg bw/day for females). It is concluded that Glyphosate technical is not carcinogenic in rats.

RMS comment:

This study is not acceptable because no core information on the test substance such as batch number or purity was given and, thus, it is not clear what was in fact tested. Even if this data would be available, the study might be considered at best supplementary because of its many additional deficiencies. No storage conditions or expiry date for the test substance was given. Mean daily dietary intake of the test substance was not given but apparently calculated by GTF afterwards. In addition, there were some deviations from OECD guideline 453, in

particular with regard to frequency of observations. Even more important, the reported total incidence of neoplasia was surprisingly low and especially the very low frequency of malignant tumours (one female control animal affected by an mammary gland adenocarcinoma among 400 animals on study) might produce doubts about suitability (i.e., sufficient vulnerability) of the rat strain used. Neither the NOAEL nor an LOAEL were proposed in the study report. In contrast to GTF opinion, the NOAEL is seen by the RMS at 3000 ppm (ca 150 mg/kg bw/day). This might be considered very conservative because it is based mainly on changes in clinical chemistry parameters without concomitant liver pathology. However, it must be taken into account that the number of animals that were subject to histopathological examination and of which organs were weighed was lower than usually required. Provided that it can be shown that glyphosate of a certain batch and precisely determined purity was tested, it would be of interest to get historical control data for tumour incidences in this rat strain and laboratory to have a better chance to assess reliability of the results with regard to carcinogenicity. In addition, the study owner should be mentioned.

4th study: [REDACTED] 2001

Reference: IIA, 5.5.2/03

Report: [REDACTED] (2001)

Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats

[REDACTED]

Data owner: Syngenta

Study No.: [REDACTED]/PR111

Date: 2001-03-15

not published, ASB2012-11488

Guidelines: OECD 453 (1981), EEC B.33 (1988), MITI (1992), US OPTTS 870.4300 (1998)

Deviations: None

GLP: yes

Acceptability: See RMS comment

Dates of experimental work: 1998-04-07 - 2000-10-16

Materials and methods

Test material:

Identification: Glyphosate acid (technical material)

Description: White solid

Lot/Batch #: P30

Purity: 97.6 % w/w

Stability of test compound: At least 2 years when stored at -20 °C.

Vehicle and/
or positive control: Diet

Test animals:

Species: Rat

Strain: Wistar (Alpk:AP_rSD)
 Source: XXXXXXXXXX
 Age: 3 weeks (on delivery)
 Sex: Males and females
 Weight at dosing: Males: 155.0 – 156.6 g (mean values); females: 136.0 – 138.4 g (mean values)
 Acclimation period: At least 10 days.
 Diet/Food: CT1 diet (Special Diet services Ltd., Essex, UK), *ad libitum*
 Water: Mains drinking water, *ad libitum*
 Housing: Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage.
 Environmental conditions: Temperature: 22 ± 3 °C
 Humidity: 30 – 70 %
 Air changes: at least 15/hour
 12 hours light/dark cycle

In life dates: 1998-04-07 to 2000-05-07

Animal assignment and treatment:

In a combined chronic toxicity and carcinogenicity study groups of 52 Wistar-derived rats per sex received daily dietary doses of 0, 2,000, 6,000 and 20,000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females).

A further twelve animals per sex were added to each group and were designated for interim kill after one year to study chronic toxicity and non-neoplastic histopathological changes.

Test diets were prepared in 60 kg batches by mixing a known amount of the test substance with 1 kg of basal diet. This pre-mix was then added to the remainder of the 60 kg batch of basal diet and mixed thoroughly. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20,000 ppm.

Clinical observations

Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study, at Week 52 and prior to termination. A functional observational battery including motor activity was conducted in Week 52 in animals allocated to the chronic toxicity assessment of the study.

Body weight

Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 15 and every two weeks thereafter until termination.

Food consumption and compound intake

Food consumption was recorded once weekly for each cage group from Week 1 to Week 14, once in week 16 and every fourth week thereafter.

Haematology and clinical chemistry

Blood was collected from 13 animals per sex and group at Week 14, 27, 53, 79 and at termination. Different animals were used for the tail vein haematology and clinical chemistry samples.

The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ -glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis

Individual urine samples were collected from the same animals as those used for haematology analyses at Week 13, 26, 52, 78 and prior to termination. The following parameters were determined: volume, abnormal colour and appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, and blood.

Sacrifice and pathology

Necropsy was conducted on all animals. The following organ weights were determined from all animals surviving to scheduled termination: adrenals, brain, gonads, heart, kidneys, liver and spleen.

Tissue samples were taken from the following organs: adrenals, aorta, bone & bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eyes (retina, optic nerve), gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, lachrymal gland, larynx, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, muscle, oesophagus, ovary, pancreas, pharynx, pituitary, prostate, rectum, salivary glands (submandibular, parotid), seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.

Statistics

All data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter using the MIXED procedure in SAS (1996). Kaplan-Meier survival estimates were calculated separately for each sex and treatment group.

The overall incidence of each tumour type was considered by comparing each treated group and the control group using Fisher's Exact Test. In addition, a test for trend with group number was performed using the Cochran-Armitage Test. Analyses were carried out for all animals, intercurrent deaths and at terminal kill.

Results and discussion

Analysis of dose formulations

The mean achieved concentrations of glyphosate acid in each dietary preparation were within 10 % of the nominal concentration and the overall mean concentrations were within 1 % of nominal.

The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20,000 ppm was satisfactory; percentage deviations were within 2 % of the overall mean for the 20000 ppm group and within 4-9 % of the overall mean for the 2000 ppm group.

The stability tests determined at 2000 and 20000 ppm showed that the test substance stability was satisfactory at room temperature and when stored at -20°C for at least 45 days which covered the period of use in the current study.

Mortality

The male groups were terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25% (criteria for termination of the study). Statistically significantly better survival was observed in males fed 20000 ppm than in the other groups ($p = 0.02$). A statistically significant overall trend was also observed for males ($p = 0.03$).

The female groups survived to scheduled termination and there were no significant differences in mortality between the groups.

The survival rates are displayed in Table B.6.5-13.

Table B.6.5-13: Survival rates during up to 104-week dietary exposure to glyphosate technical

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Week 13	0.98	1.00	1.00	1.00	0.98	0.98	1.00	1.00
Week 26	0.95	1.00	1.00	1.00	0.98	0.98	1.00	1.00
Week 39	0.94	1.00	1.00	1.00	0.97	0.98	1.00	1.00
Week 52	0.91	1.00	0.97	1.00	0.97	0.98	0.98	0.98
Week 56	0.89	1.00	0.93	1.00	0.93	0.98	0.98	0.98
Week 60	0.87	1.00	0.92	1.00	0.91	0.97	0.98	0.97
Week 64	0.87	1.00	0.90	0.98	0.91	0.95	0.98	0.97
Week 68	0.87	0.94	0.88	0.96	0.87	0.95	0.98	0.95
Week 72	0.85	0.94	0.84	0.96	0.85	0.93	0.97	0.91
Week 76	0.81	0.94	0.80	0.92	0.82	0.89	0.97	0.91
Week 80	0.73	0.88	0.78	0.87	0.72	0.89	0.89	0.83
Week 84	0.69	0.85	0.67	0.83	0.63	0.89	0.85	0.83
Week 88	0.64	0.81	0.57	0.81	0.59	0.83	0.77	0.81
Week 92	0.56	0.79	0.50	0.81	0.53	0.81	0.71	0.80
Week 96	0.50	0.73	0.46	0.73	0.53	0.77	0.66	0.72
Week 100	0.40	0.69	0.44	0.63	0.42	0.77	0.56	0.66
Week 104	—*	0.62	—*	0.56	—*	0.77	—*	0.57

* Terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25% (criteria for termination of the study).

Clinical observations

At 20000 ppm there was a treatment related increase in the incidence of red-brown staining of tray papers, particularly in males.

There were no other treatment related clinical observations.

There were also no treatment-related effects noted in the functional observational battery.

Body weight

The body weights of the animals fed 20000 ppm glyphosate acid were statistically significantly lower than controls throughout the study. The maximum reduction from control values was approximately 5 % for males and 8 % for females.

There were no treatment related effects in animals fed 2000 or 6000 ppm glyphosate acid.

Food consumption and compound intake

Food consumption was lower throughout the first year of the study in animals fed 20000 ppm glyphosate acid. In females the difference was statistically significant over the first 11 weeks (with a maximum reduction of approximately 5%) and again in weeks 40-56 (with a maximum reduction of 6%). In males, the difference was statistically significant over most of the first 6 months with a maximum reduction of 6%.

The group mean achieved doses are summarised below.

Table B.6.5-14: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	2000	121	145
3 (mid)	6000	361	437
4 (high)	20000	1214	1498

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.

Ophthalmoscopy

There were no treatment-related effects observed.

Haematology and clinical chemistry

Haematology

Minor variations from control values were obtained for most parameters but showed no consistency and were confined to intermediate time points and/or dose groups and were considered not to be treatment-related. An increased haemoglobin concentration and decreased platelet count was seen in all female treated groups at the interim kill but, in the absence of any apparent dose-response or effects at other time points, these variations from mean control values are considered not to be treatment-related (see Table B.6.5-15).

Table B.6.5-15: Haemoglobin and platelet count

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Haemoglobin (g/dL)								
Week 14	15.9	15.7	16.0	15.5	16.0	15.9	15.8	15.0*
Week 27	15.5	15.7	15.8	15.8	15.8	15.7	15.9	15.6
Interim Kill	14.7	14.4	14.4	15.1**	14.3	14.9*	14.4	15.0*
Week 53	16.1	15.9	15.7*	15.9	15.5**	15.9	15.9	15.8
Week 79	15.9	15.9	15.2	15.8	15.5	16.0	15.4	15.5
Week 105	13.3	14.3	12.9	14.1	13.1	13.8	13.6	14.2
Platelet count ($\times 10^9/L$)								
Week 14	885	911	897	877	892	910	847	948
Week 27	903	909	871	868	917	858	880	830*
Interim Kill	889	821	895	761*	888	740**	860	764*
Week 53	911	842	977	794	911	754	865	814
Week 79	963	854	993	796	950	817	935	855
Week 105	1015	780	980	783	988	750	877	846

* $p < 0.05$; ** $p < 0.01$ **Clinical chemistry**

In rats fed 20000 ppm glyphosate acid, increases in plasma alkaline phosphatase were present until Week 79 (Table B.6.5-16). Increases in alanine aminotransferase activities were present consistently in males until Week 79 and in females in Weeks 14, 79 and 105. Increased total bilirubin was also present in these males throughout the study and increased plasma aspartate aminotransferase activity was present in males at the interim kill. Plasma triglycerides and cholesterol levels were reduced (from Weeks 14-53 and Weeks 53 onwards, respectively) in males.

In animals fed 6000 ppm, there were small increases in alkaline phosphatase activity over the first year of the study and variable increases in plasma alanine aminotransferase activity at intermediate time points throughout the study.

Plasma creatinine values were lower in all treated female groups at Week 27 and in females receiving 6000 and 20000 ppm at Week 14, but in the absence of any effects later in the study, this is considered to be of no toxicological significance.

Other minor variations from mean control values were confined to intermediate dose groups or time points and/or showed no dose response, and so were considered not to be treatment-related.

Table B.6.5-16: Clinical chemical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Alkaline Phosphatase (IU/L)								
Week 14	234	156	246	177	284**	245**	387**	266**
Week 27	196	121	219	136	239**	166**	327**	203**
Interim Kill	230	82	244	102	269	123*	306**	144**
Week 53	231	92	249	117*	277**	152**	357**	172**
Week 79	208	114	254*	131	244	181**	353**	178**
Week 105	184	144	205	129	218	158	280	173
Alanine Aminotransferase (IU/L)								

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 14	94.9	81.9	103.5	92.5	121.8**	103.9*	143.4**	104.7*
Week 27	91.8	99.5	95.9	113.8	116.8	132.7*	125.9*	101.8
Interim Kill	77.6	83.4	84.0	82.8	97.7	113.2*	123.3**	95.9
Week 53	84.2	90.1	99.8	108.2	103.5	121.5*	133.8*	114.0
Week 79	69.2	90.0	81.2	97.2	102.4**	110.6	105.9**	116.0*
Week 105	64.1	83.5	58.6	78.6	63.9	78.9	82.7	108.2**
Total Bilirubin (µmol/L)								
Week 14	1.23	2.00	1.23	1.92	1.46	2.00	1.85**	2.46*
Week 27	2.08	2.31	2.31	2.08	2.31	2.08	2.62**	2.23
Interim Kill	2.09	2.50	1.91	2.42	2.18	2.58	2.67**	2.64
Week 53	2.62	2.54	2.46	2.31	2.92	2.46	3.46**	3.15**
Week 79	2.46	2.92	2.92	2.31	2.85	2.38	3.15**	3.08
Week 105	1.75	1.19	2.29	1.04	1.67	1.77	2.54	1.40
Aspartate Aminotransferase (IU/L)								
Week 14	107.9	104.5	113.5	112.6	129.2	124.0	148.0*	114.3
Week 27	110.5	156.8	114.8	185.5	138.0	208.4	141.3	148.3
Interim Kill	90.0	117.8	91.5	109.0	110.4	149.3	132.0*	131.5
Week 53	111.8	151.9	124.8	194.4	130.2	219.1*	160.7	214.8*
Week 79	88.2	156.0	102.7	129.2	130.0	177.7	112.2	197.0
Week 105	75.8	130.7	81.4	102.8	78.4	121.8	92.8	168.5
Plasma Triglycerides (mmol/L)								
Week 14	1.33	1.03	1.48	0.96	1.43	0.96	1.11*	0.94
Week 27	1.40	1.18	1.42	1.22	1.38	0.95*	1.14*	1.09
Interim Kill	1.65	1.00	2.07	1.13	2.09	1.07	1.45	0.99
Week 53	1.53	1.62	1.55	1.75	1.50	1.39	1.15*	1.39
Week 79	1.90	2.15	1.96	2.77	1.67	2.26	1.42	2.31
Week 105	1.83	3.26	1.81	3.58	1.94	3.02	1.67	2.82
Cholesterol (mmol/L)								
Week 14	2.40	2.66	2.51	2.62	2.48	2.80	2.54	2.71
Week 27	2.92	3.19	3.02	3.24	3.18	3.13	2.98	3.15
Interim Kill	4.74	2.69	5.05	2.95	4.83	2.98	3.89*	3.01
Week 53	5.09	3.56	4.57	3.49	5.15	3.45	4.06**	3.66
Week 79	6.87	4.26	6.30	4.64	5.81*	3.92	5.20**	3.96
Week 105	6.76	4.44	7.22	4.54	7.79	4.13	5.72*	4.11
Plasma Creatinine (µmol/L)								
Week 14	58.5	61.4	59.9	59.6	57.2	59.0*	56.8	58.6**
Week 27	60.8	62.7	61.2	60.3*	59.4	60.5*	58.4*	58.2**
Interim Kill	55.8	53.6	58.0	51.8	56.5	52.3	56.6	50.9
Week 53	61.0	58.8	61.5	59.5	62.5	58.1	60.5	58.2
Week 79	80.7	62.7	85.9	59.2	86.2	62.8	66.4	61.8
Week 105	79.1	50.9	80.8	51.4	79.2	53.5	66.2	50.7

* p < 0.05 ** p < 0.01

Urinalysis

Urinary pH was lower throughout the study in males fed 20000 ppm glyphosate acid (Table B.6.5-17). Moreover, in the same dose group an increased incidence and severity of blood/red blood cells was present in males and, to a lesser extent, in females.

There were no other treatment related findings in the urinalysis.

Table B.6.5-17: Urinalytical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Urine pH								
Week 13	6.85	6.00	6.77	6.00	6.92	6.08	6.30**	5.85
Week 26	6.77	5.77	6.69	5.85	6.69	6.00	6.15**	5.77
Week 52	6.85	6.15	6.85	6.23	6.85	6.31	6.15**	5.92
Week 78	6.54	6.38	6.28	6.77	6.15	6.46	5.69**	6.00
Week 98	6.08	—	6.00	—	6.00	—	5.85	—
Week 104	—	6.00	—	6.08	—	6.15	—	6.00

** p < 0.01; NEG: negative, +: very few (1 or 2); ++: few; +++: many

Necropsy

Gross pathology

Treatment-related macroscopic findings were seen in males fed 20000 ppm and/or 6000 ppm in the kidneys, liver, prostate and testes. These findings consisted of a minor increase in incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes.

Additional findings were not considered to be treatment related.

Organ weights

Significant lower relative adrenal gland weight was noted at the interim kill in females fed 20000 ppm and 6000 ppm glyphosate acid. Furthermore, the liver weight was significantly lower at the interim kill in males fed 20000 ppm glyphosate acid.

There were no other significant and dose-related effects on organ weights.

Histopathology

A minor increase in the incidence but not severity of proliferative cholangitis in the liver was present in males fed 20000 ppm glyphosate acid at interim and terminal kill (see Table B.6.5-18).

Moreover, in males fed 20000 ppm glyphosate acid an increased incidence of hepatitis and periodontal inflammation was observed. The incidence of prostatitis was higher than the control group in all treated males and there was a decrease in the incidence of tubular degeneration of the testis in males fed 20000 ppm glyphosate acid. The incidence of prostatitis was within historical background levels in all treated groups but, as the control value in this study was low, the relationship to treatment at the high dose level cannot be entirely dismissed.

The main changes in interim and terminal kill males and, to a lesser extent, females fed 20000 ppm glyphosate acid, were observed in the kidney. These changes consisted of slight increased incidence of papillary necrosis with varying degrees of mineralisation of the papilla and/or transitional cell hyperplasia. There was also a very small increased incidence of papillary mineralisation only (males and females fed 20000 ppm glyphosate acid) and transitional cell hyperplasia alone (20000 ppm males only).

All other observed differences in the incidence of findings either fall within the historical background level or are considered to be unrelated to the treatment with glyphosate acid.

Table B.6.5-18: Summary of histopathological findings

Finding	Dietary concentration of glyphosate (ppm)									
	Males (n=64)					Females (n=64)				
	Historical Control	0	2000	6000	20000	Historical Control	0	2000	6000	20000
<i>Liver</i>										
Proliferative cholangitis	-	56	57	55	64	-	55	58	59	61
Hepatitis	4.7 [2 - 8]	8	6	9	13	-	6	7	4	6
<i>Kidney</i>										
Papillary necrosis	0.4 [0 - 2]	0	1	0	14	-	0	1	2	5
Transitional cell hyperplasia	-	2	3	0	5	-	-	1	0	1
<i>Prostate</i>	23.4									
Prostatitis	[13 - 35]	13	22	23	37	-	-	-	-	-
<i>Testis</i>										
Unilateral tubular degeneration	-	18	13	18	5	-	-	-	-	-
Periodontal inflammation	-	25	27	23	42		18	24	32	28

n = number of animals per group

Historical control (mean and [range])

Neoplastic changes

There was no evidence of carcinogenicity and no differences between the groups in tumour incidence.

Conclusion by the Notifiers

Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 24 month is 6000 ppm (corresponding to 364 mg/kg bw/day in males and 437 mg/kg bw/day in females). It is concluded that glyphosate technical is not carcinogenic in rats.

RMS comment:

The study is considered acceptable. We agree with the description of the study and its findings and support the conclusions including the NOAEL. It was surprising that the salivary gland findings reported by Milburn (1996, TOX2000-1998) were not confirmed although the study was run in the same laboratory employing rats of the same strain. No further remarks.

5th study: [REDACTED] (2009)**Reference:**

IIA, 5.5.2/08

Report:

[REDACTED] (2009)

Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat

Study No.: 2060-0012

Date: 2009-04-23, amended 2009-05-08

not published, ASB2012-11490

Guidelines:

OECD 453 (1981), JMAFF Guideline 2-1-16 (2005), US OPTTS 870.4300 (1996)

Deviations:

None

GLP:

yes

Acceptability: See RMS comment

Dates of experimental work: 2005-09-01 - 2008-03-19

Materials and methods

Test material:

Identification: Glyphosate Technical

Description: White crystalline solid

Lot/Batch #: H05H016A

Purity: 95.7 % w/w

Stability of test compound: No data

Vehicle : Diet

Test animals:

Species: Rat

Strain: Wistar Han Crl:WI

Source:

Age: 5 – 6 weeks

Sex: Males and females

Weight at dosing: Males: 112 – 183 g, females: 98 – 150 g

Acclimation period: At least ten days

Diet/Food: Rat and Mouse SQC Ground Diet No.1 (BCM IPS Ltd., London, UK), *ad libitum*

Water: Mains drinking water, *ad libitum*

Housing: Initially in groups of three per sex in polypropylene solid-floor cages.

Environmental conditions: Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: at least 15/hour
12 hours light/dark cycle

In life dates: 2005-09-01 to 2007-08-31

Animal assignment and treatment:

In a combined chronic toxicity and carcinogenicity study groups of 51 Wistar rats per sex received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 95.0, 316.9 and 1229.7 mg/kg bw/day) Glyphosate technical. To ensure that a received dose of 1000 mg/kg bw/day overall was achieved, the highest dose level was progressively increased to 24000 ppm.

In addition, three satellite groups with 15 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes. The satellite control group with 12 rats per sex served as veterinary control. The animals were to be used for investigations should any health problems have developed with study animals. No such problems occurred and therefore the observations of these animals have not been included in the report.

Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet for 19 minutes at a constant speed. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.

The stability and homogeneity of the test substance in the diet was determined in an in-house stability study. The homogeneity and achieved concentrations of the test substance preparations was determined at monthly intervals until Week 26, and in 3-month intervals thereafter.

Clinical observations

Rats were examined for toxic signs, ill-health or behavioural changes once and for pre-terminal deaths twice a day. A routine clinical observation session including veterinary examination was made weekly, including palpation for new or existing masses. Ophthalmic examination was done at the start of the study in all satellite animals and at Week 50 in ten satellite animals per sex of the control and high dose group. Prior to treatment and at weekly intervals thereafter all satellite animals were observed for behavioural toxicity.

Body weight

Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13 and every four weeks thereafter until termination as well at terminal kill.

Food consumption and compound intake

Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently for one week in each four weeks until termination.

Water consumption

Water intake was observed daily, for each cage group, by visual inspection.

Haematology and clinical chemistry

Haematological examinations were performed on ten animals per sex from the satellite and main groups at 3, 6 and 12 months. Further haematological investigations were performed on 20 animals per sex from the main groups at 18 and 24 months. The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, prothrombin time, and activated partial thromboplastin time.

Blood chemical investigations were performed on ten animals per sex from the satellite groups at 6 and 12 months and from the main groups at 18 and 24 months. The following parameters were determined: urea, glucose, total protein, albumin, albumin/globulin ratio, sodium, potassium, chloride, calcium, inorganic phosphorus, ASAT, ALAT, alkaline phosphatase, creatinine, total cholesterol, total bilirubin, and cholinesterase.

Urinalysis

Urinalytical investigations were performed on ten animals per sex from satellite groups at 3, 6 and 12 months and from main groups at 18 and 24 months. The following measurements were made: specific gravity, volume, pH, protein, glucose, ketones, blood, urobilinogen, reducing substances and microscopic examination of sediment.

Sacrifice and pathology

Necropsy was conducted for all animals surviving until study termination (main groups: 104 weeks; satellite groups: 52 weeks) as well for all animals found dead or killed *in extremis*.

The following organ weights were determined from 10 rats per sex and main group and from all satellite animals: adrenals, brain, gonads, heart, kidneys, liver, spleen and thymus.

Tissue samples were taken from the following organs: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions including palpable masses, head (pharynx, nasopharynx, paranasal sinuses), heart, Harderian gland, ileum (incl. Peyer's patches), jejunum, kidneys, liver, lungs (with bronchi), lymph nodes (cervical and mesenteric), mammary gland, muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands (submaxillary), sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina. A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.

Histopathological examination was initially carried out on all tissues collected from control and high dose groups; all pre-terminally dead and moribund sacrificed rats and on all lesions and palpable masses of the terminally sacrificed rats from the low and mid dose groups.

Since there were no indications of treatment-related bone marrow changes, examination was subsequently extended to the remaining treatment groups.

Statistics

Where appropriate quantitative data was analysed by the ProvantistTM Tables and Statistics Module. For each variable, the most suitable transformation of the data was found; the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANCOVA and Bartlett's test. The transformed data was analysed to find the lowest treatment level that shows a significant effect, using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response is found, but the data shows non-homogeneity of means, the data will be analysed by a stepwise Dunnett (parametric) or Steel (non-parametric) test to determine significant differences from the control group. Finally, if required, pair-wise tests are performed using the Student t-test (parametric) or the Mann-Whitney U test (non-parametric).

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes.

1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of I or greater.
2. Kruskal-Wallis one-way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

Results and discussion

Analysis of dose formulations

Stability assessment demonstrated that the test material preparations in the diet were stable for at least six weeks.

Analyses for achieved concentrations showed that the diet preparations were within an acceptable range. On one occasion the achieved concentrations of the low, mid and high-dose group were 79%, 83%, and 87%, respectively. At week 2 the concentration in the mid dose group was 112%. However, these isolated deviations from the nominal range were still considered to be acceptable.

Mortality

No significant treatment-related effects on mortality were observed during the study.

The numbers of pre-terminal deaths in the main group are displayed below:

Table B.6.5-19: Cumulated mortalities after 104-week dietary exposure to glyphosate technical

	Dose group (ppm)			
Sex	0	1500	5000	15000-24000
Male	12	14	13	6
Female	14	17	15	12

Clinical observations

No significant treatment-related clinical observations occurred during the study.

There were no treatment-related effects on behavioural assessments, functional performance tests or sensory reactivity assessments observed.

Body weight

There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

Food consumption and compound intake

There were no treatment-related effects on food consumption or food efficiency for either sex noted during the study.

The group mean achieved doses are summarised below.

Table B.6.5-20: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)		Mean achieved dose level (mg/kg bw/day)		
			Males	Females	Overall mean
1 (control)	0				
2 (low)	1500		85.5	104.5	95.0
3 (mid)	5000		285.2	348.6	316.9
4 (high)	15000	Week 1-11	1077.4	1381.9	1229.7
	17000	Week 12-15			
	19000	Week 16-26			
	21000	Week 27-39			
	24000	Week 40-104			

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 95.0, 316.9 and 1229.7 mg/kg bw/day for 1500, 5000, and 15000-24000 ppm, respectively. The mean intake values represent the combination of satellite and main group values.

Water consumption

There were no treatment-related effects on water consumption during the study.

Ophthalmoscopy

There were no treatment-related effects observed.

Haematology and clinical chemistry

Haematology

All variations were considered to be incidental and unrelated to treatment because of the lack of either a true dose response, a consistent change throughout the study, a lack of progression of change with time and/or lack of concomitant effect in both sexes.

Clinical chemistry

At the highest dose level there was an increase in alkaline phosphatase activity for satellite group males and females compared with controls at 6 and 12 months. Main group males were also affected at 18 months. Values for all alkaline phosphatase activity values are presented as follows:

Table B.6.5-21: Alkaline phosphatase activity (IU/L)

	Dose level							
	Control		Low		Intermediate		High	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Month 6 (Satellite)	87.8	49.6	94.5	62.9	103.4	62.0	128.5**	91.9**
Month 12 (Satellite)	87.7	46.1	96.5	59.7	116.3*	58.1	140.2**	91.3**
Month 18 (Main)	93.3	65.7	110.5	55.8	110.9	70.9	125.0*	92.7
Month 24 (Main)	107.2	66.0	98.8	58.5	101.0	81.7	111.9	86.8

* $p < 0.05$; ** $p < 0.01$

The magnitude of the effect does not appear to increase with age plus the lack of a consistent effect for females does suggest this to be of limited toxicological importance.

At the 18 month evaluation there was an increase in plasma electrolytes for both sexes. Sodium and chloride values for males and females and potassium values for males only were increased compared with controls. Female calcium levels were lower than controls. These elevations/decrements were also observed at lower dose levels but were not seen in a dose related trend. In addition at the 12 month evaluation for satellite females a lower sodium value was seen for females. Values for all calcium and chloride values are presented as follows:

Table B.6.5-22: Calcium and chloride values (mmol/L)

	Dose level							
	Control		Low		Intermediate		High	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Calcium								
Month 6 (Satellite)	2.587	3.693	2.701	3.752	2.617	3.637	2.508	3.604*
Month 12 (Satellite)	2.530	2.602	2.543	2.587	2.458	2.475	2.514	2.483
Month 18 (Main)	2.231	2.775	2.523	2.645*	2.656	2.554**	2.598	2.468**
Month 24 (Main)	2.431	2.293	2.487	2.396	2.511	2.288	2.297	2.347
Chloride								
Month 6 (Satellite)	107.7	105.8	107.1	106.1	107.0	106.1	108.5	106.7
Month 12 (Satellite)	105.6	103.9	105.1	104.8	104.3	104.7	105.9	104.2
Month 18 (Main)	103.3	101.8	105.8**	104.2**	105.8**	106.4**	107.6**	107.8**
Month 24 (Main)	104.5	103.4	104.4	103.1	104.3	102.2	105.4	102.8

* $p < 0.05$; ** $p < 0.01$

At intermediate level similar findings to the highest dose level were seen for plasma electrolytes at the 18 month evaluation. A slight increase in alkaline phosphatase activity was seen for satellite group males at 12 months. At the low dose level there was a similar effect on

the plasma electrolytes for both sexes at the 18 month evaluation of main group animals. Whilst these observations were seen at the highest dose level, the lack of dose response or the effect being limited to one sex does make the toxicological significance questionable.

All other differences were isolated in their finding and are therefore not toxicologically relevant.

Urinalysis

There were no treatment-related effects observed.

Necropsy

Gross pathology

There were no treatment-related macroscopic findings observed during the study period.

Organ weights

No effects on organ weight values were observed.

Histopathology

Adipose infiltration of the bone marrow was seen for the majority of animals examined, with both sexes being more or less equally affected in terms of incidence and severity. However, greater effects were seen among male rats dosed at the highest level and this attained statistical significance for terminal kill animals. This data indicates the possibility of myeloid hypoplasia as a consequence of treatment. However, given the normal variability of this condition and the influence of other pathological conditions upon marrow cellularity in ageing rats, the effect was not altogether convincing but cannot be dismissed. A similar effect was not seen among male rats in the remaining treatment groups but among premature deaths for animals of both sexes at the intermediate level and only low-dosed females. However, the variable duration of exposure and significant background pathology for premature death animals further negates this as an effect of treatment upon marrow cellularity for female rats.

Moreover, at the highest dose level there was a significant difference in the site of mineral deposition within the kidneys compared with controls. Pelvic mineralisation was commonly seen in both sexes and was more prevalent among female rats; however corticomedullary mineralisation was seen in female rats only. Nephrocalcinosis in rats is generally considered to be related to diet and hormonal status. There was a lower incidence of pelvic/papillary deposition and an increase in the corticomedullary deposition. At the same time there was a reduction in the incidence of renal pelvic hyperplasia in both sexes; which is considered to be a consequence of the decreased mineral deposition.

The effects on pelvic and corticomedullary mineralisation, and hyperplasia of the pelvic/papillary epithelium were confined to high dose animals with no indication of a similar effect at any other treatment level for either sex.

No other treatment-related changes were observed.

Neoplastic changes

No significant effects associated with tumour development were observed.

Conclusion by the Notifiers

Based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 24000 ppm (corresponding to 1229.7 mg/kg bw/day for combined sexes). It is concluded that Glyphosate technical is not carcinogenic in rats.

RMS comment:

The study is acceptable. In principle, the conclusions are agreed with but the NOAEL is seen rather at the mid dose level of 5000 ppm (equal to 285 mg/kg bw/day in males) because of minor effects that were observed at the highest dose level. These effects comprised a by up to 9 % lower body weight gain in male rats, a transient increase in alkaline phosphatase activity (confirming the same findings in many other studies with glyphosate), fatty infiltration of the bone marrow that might be indicative of hypoplasia and the kidney findings of equivocal relevance. In addition, there were some histological skin changes that resemble those reported by [REDACTED] (1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487). They comprised a slightly higher incidence of areas of necrosis or giant cell reaction to keratin and a non-significant increase in keratoacanthoma in high dose males (occurring in 6/51 animals as compared to 2/51 in the control group, 3/51 in the low and 0/51 in the mid dose groups; incidence in females always 0). However, the assessment of glyphosate as non-carcinogenic in this study is not doubted.

B.6.5.1.2 Previously known long-term studies in rats (already subject to EU evaluation)

1st study: [REDACTED] 1981

Reference: IIA, 5.5.2/05

Report: [REDACTED] (1981)

A Lifetime Feeding Study
of Glyphosate (ROUNDUP Technical) in Rats

Data owner: Monsanto

Study/Project No.: 77-2062

Date: 1981-09-18

not published, TOX2000-595

Guidelines: Not stated. In general accordance with OECD 453 (1981)

Deviations: None

GLP: no

Acceptability: See RMS comment

Dates of experimental work: In-life: 1978-07-12 to 1980-09-04

Materials and methods

Test material:

Identification: Glyphosate acid (Round-up technical material)

Description: Fine White powder

Lot/Batch #: XHJ-64

Purity: 98.7 % w/w

Stability of test compound: At least 45 days when stored at -20 °C.

Vehicle and/
or positive control: Diet

Test animals:

Species: Rat
 Strain: Sprague-Dawley CD
 Source: [REDACTED]
 Age: 28 days (on delivery), 41 days at initiation of delivery
 Sex: Males and females
 Weight at dosing: Males: 155.0 – 156.6 g (mean values); females: 136.0 – 138.4 g (mean values)
 Acclimation period: 12 days.
 Diet/Food: Standard laboratory diet (Purina Lab Chow) *ad libitum*.
 Freshly prepared weekly
 Water: Mains automated water system (Elizabethtown Water Company), *ad libitum*
 Housing: Individually in elevated stainless steel cages.
 Environmental conditions: Temperature: Monitored but values are not stated
 Humidity: not stated
 Air changes: not stated
 12 hours light/dark cycle

In life dates: 12-07-1978 to 04-09-1980

Animal assignment and treatment:

In a chronic toxicity and carcinogenic study, groups of 50 male and 50 female Sprague-Dawley rats were administered in the diet glyphosate (Round-up technical) at concentrations of 0, 30, 100 and 300 ppm for the first week and at concentrations of 0 (control) 3.05, 10.30 and 31.49 mg/kg bw/day for the males, and 0 (control), 3.37, 11.22 and 34.02 mg/kg bw/day for the females for 26 months. Males received treatment for 775 - 776 days and females 784 - 785 days before termination.

A 0.5 oz. sample of the test substance was taken at weeks 1, 11, 16, 24, 36, 48, 60, 72, 84 and 96 and submitted to the sponsor for analysis. Samples of control feed and feed for each dose level were collected for analysis.

Observations

Rats were examined for mortality and signs of toxicity twice daily. Detailed physical and clinical examinations were performed weekly and included palpations for tissue masses. Ophthalmic examination was done in all animals at the start of the study, at Week 52 and prior to termination. A functional observational battery including motor activity was conducted in Week 52 in animals allocated to the chronic toxicity assessment of the study.

Body weight

Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 14 and every two weeks thereafter until termination.

Food consumption and compound intake

Food consumption was recorded pre-test, once weekly for from Week 1 to Week 14 and every second week thereafter until termination.

Water consumption

Water consumption was investigated during the 18 and 24 months of treatment over 2-three day periods in 10 animals/sex/dose group.

Laboratory investigations

Haematology and clinical chemistry investigations were performed on 10 males and 10 females of each dose group during Months 4, 8, 12, 18 and 24 months. Blood was obtained via venipuncture of the orbital sinus (retrobulbar venous plexus) under light ether anaesthesia. Animals were selected randomly; the same animals were used at all intervals when feasible. Rats were fasted overnight prior to blood collections and were not dosed until after samples were collected.

Haematological parameters investigated included haematocrit, haemoglobin, erythrocyte count, platelet count, total leukocyte count and differential leukocyte count. Clinical chemistry parameters were alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic acid dehydrogenase, blood urea nitrogen, fasting glucose, albumin, globulin, albumin/globulin ratio, total bilirubin and direct bilirubin, triglycerides, cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride. However as a result of a technician error, potassium was not evaluated at months 8 and 12 and inorganic phosphorus was evaluated at months 8 and 12 only.

Urinalysis was performed during the same months as for haematology and clinical chemistry except at 8 months and the parameters reported included gross appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, and blood and microscopic analysis.

Sacrifice and pathology

Necropsy was conducted on all animals which died prematurely or were killed at termination as scheduled. Organ weights were determined for all animals surviving to scheduled termination and included the adrenals, brain, gonads, heart, thyroid, kidneys, liver, pituitary and spleen.

Samples from organs and tissues including the adrenals, aorta, blood smears, bone & bone marrow (costochondral junction), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eyes (retina, optic nerve), gross lesions including palpable masses, Harderian gland, heart, intestines (Including the caecum, colon, duodenum, ileum and jejunum), kidneys, lachrymal gland, larynx, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, muscle, oesophagus, ovary, pancreas, pharynx, pituitary, prostate, rectum, salivary glands (submandibular, parotid), seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and vagina.

Statistics

Parameters analyzed statistically were bodyweight, food consumption, haematology and clinical chemistry values, terminal organ and body weights, organ/body weight ratios and organ/brain weight ratios.

Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was

used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control. A statistical test for trend in the dose levels was also performed. In the parametric case (i.e. equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used. The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

Results and discussion

Analysis of dose formulations

Results of diet analyses were not reported.

Mortality and clinical signs

There was no significant difference between the control and treated both sexes with regard to the survival rate during the course of this study. Survival was approximately 80-90% through Month 20 of the study for all groups. Thereafter, significant reductions in the number of surviving animals occurred in all groups in roughly an equivalent fashion, culminating in the termination of the study at Month 26. At this time, survival had decreased to 30% in the low dose males and the high dose females, requiring that the study be terminated to insure a sufficient number of animals at the terminal necropsy. At 24 months, survival levels equalled or exceeded 50%, which is comparable to historical control data for rats of this strain.

The survival rates are displayed in Table B.6.5-23.

There were no treatment-related signs of toxicity and physical findings observed including alopecia, excessive lacrimation, nasal discharge and rales were present in all groups without a treatment-related trend.

Table B.6.5-23: Survival rates during up to 26-month dietary exposure to glyphosate technical (%)

	Dose group (mg/kg bw/day)							
	Males				Females			
	0	3.05	10.30	31.49	0	3.37	11.22	34.02
Month 20	74	86	86	96	92	88	88	76
Month 24	44	56	46	66	52	62	64	48
Month 26	30	52	32	52	36	46	60	30

Functional observations

A functional observational battery of tests was not performed. It is not considered to affect the validity of this study.

Body weight

There were no statistically significant differences in mean body weights in males. During part of the growth period, a slight but consistent trend toward reduced body weights in the treated males was evident. The maximum decrease was approximately 6% in high dose males. Thereafter, this difference decreased resulting in little difference in mean body weights between groups at termination. Because this effect was slight and not evident at termination of the study and did not affect survival, it is not considered to be toxicologically significant. In females, no statistically significant difference in mean body weights was observed in

treated animals compared to the controls up to Month 19 of the study. However, for the following 2 months, the treated groups showed statistically significant reductions in mean body weights as compared to the control, although not in a dose-related fashion. The magnitude of the reduction ranged between 10-15% with the greatest difference evident in the low and mid-dose group. Thereafter, the treated females gained weight relative to the control group resulting in nearly identical group mean body weights at termination. The absence of a dose-response relationship in this observation suggests the finding was most likely due to biological variation which is evident from the standard deviation of the mean body weights for all dose groups. The body weight changes are noted to have occurred well after the main growth phase in both sexes and lacked a dose-response relationship in females. The top dose was only statistically significantly reduced compared with controls during weeks 92 and 94 when body weight reduction was approximately 11.5% and 11% respectively; however at the next measurement during Week 96 body weight reduction compared with controls was only 5.7 %.

Food consumption and compound intake

Occasional statistically significant differences were noted in the treated animals of both sexes relative to their respective controls. However, these changes in mean food consumption values were slight and occurred sporadically and showed no treatment-relationship.

The target concentrations for treatment as administered in the first were 0 (control), 30, 100 and 300 ppm which corresponded to approximate compound intakes of 0, 3.05, 10.30 and 31.49 mg/kg bw/day for males and 0, 3.37, 11.22 and 34.02 mg/kg bw/day.

Water consumption

There were no treatment-related effects observed.

Laboratory investigation

Haematology

Haematology data did not indicate any toxicologically significant differences in the findings for both sexes for any of the parameters evaluated. All mean data were within the normal physiological range for the laboratory rat. The few statistically significant differences noted appeared to be due to random variation as no consistent treatment-related pattern was evident. Thus, haematological parameters were unaffected by the treatment of glyphosate.

Clinical chemistry

Clinical chemistry parameters for both males and females were within the normal physiological range and did not deviate significantly in a treatment-related manner from controls. Occasional statistically significant differences were noted, but these appear to be due to random fluctuation, as no treatment-related pattern emerged.

Urinalysis

Urinalysis parameters did not show any significant differences were between treated and control groups. Occasional values outside the normal range were found; however, these values occurred sporadically exhibiting no consistent pattern..

Necropsy

Gross pathology

There were no treatment-related gross pathological findings.

Organ weights

There were no statistically significant differences noted in the terminal organ weights, organ/body weight ratios and organ/brain weight ratios of the treated animals compared with their respective controls.

Histopathology

Neoplastic changes

The most common tumours were found in the pituitary in both sexes of both control and treated animals

In the females, mammary gland tumours were the next most common neoplasm found. In general, the incidence of all neoplasms observed in the treated and control animals were to a similar degree, or occurred at low incidence such that a treatment-related association could not be made (Table B.6.5-24).

Table B.6.5-24: Summary of critical tumour findings in 26-month dietary study with glyphosate technical

	Dose group (mg/kg bw/day)							
	Males				Females			
Dose Groups	0	3.05	10.3	31.49	0	3.37	11.22	34.02
Pituitary tumours								
Adenomas	16/48	19/49	20/48	18/47	34/48	29/48	31/50	26/49
Carcinomas	3/48	2/49	3/48	1/47	8/48	7/48	5/48	12/49
Combined	19/48	21/49	23/48	19/47	42/48	36/48	36/50	38/49

However, the incidence of interstitial cell tumours of the testes in male rats in both the scheduled terminal sacrifice animals as well as for all animals suggested a possible treatment-related finding and was presented along with the most recent historical control data at the time of the study for comparison. It was noted that at 12 months the incidence of interstitial tumours was near zero however in animals aged 24-29 months at necropsy, the incidence increased to approximately 10%. The historical control data for chronic toxicity and carcinogenicity from 5 studies terminated at 24-29 months showed background levels of interstitial cell tumours comparable to that found at the highest dose in the study. The incidence of interstitial cell hyperplasia did not provide evidence of a preneoplastic lesion.

Table B.6.5-25: Summary of the interstitial cell tumour findings in the testes of rats after 26-month dietary exposure to glyphosate technical

Parameter	Dose group (mg/kg bw/day)*			
	0	3.05	10.3	31.49
Interstitial cell tumour				
Terminal sacrifice	0/15 (0%)	2/26 (7.7%)	1/16 (6.3%)	4/26 (15.4%)
All Animals	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50 (12%)
Interstitial cell hyperplasia				
Terminal sacrifice	1/15 (6.7%)	1/26 (3.8%)	0/16 (0%)	0/26 (0%)
All Animals	1/50 (2%)	1/50 (2%)	1/50 (2%)	0/50 (0%)

*number of animals affected / total number of animals examined

(): Percentage

Table B.6.5-26: Summary of the concurrent historical control data for interstitial cell tumours in the testes of in chronic toxicity studies

Parameter	Study				
	1	2	3	4	5
Terminal Sacrifice	4/65 (6.2%)	3/11 (27.3%)	3/26 (11.5%)	3/24 (12.5%)	3/40 (7.5%)
All Animals	4/116 (3.4%)	5/75 (6.6%)	4/113 (3.5%)	6/113 (5.3%)	5/118 (4.2%)

*number of animals affected / total number of animals examined

(): Percentage

The investigators noted that at terminal sacrifice, the incidence in the present study was 15.4% (4/26), while the range in control animals from 5 contemporary studies (historical controls) was 6.2% (4/65) to 27.3% (3/11) with an overall mean value of 9.6% (16/166). When all animals on test are included, the incidence for the Group IV males was 12% (6/50) compared to a range of 3.4% (4/116) to 6.7% (5/75) with a mean of 4.5% (24/535). Therefore, this comparison suggests an incidence of this tumour in the Group I males which is slightly lower (0%), and an incidence in the Group IV males which is slightly higher than recent historical control data. Although an effect on the incidence of this tumour due to the administration of the test substance cannot be ruled out, the data suggests that the incidence in treated rats is within the normal biological variation observed for tumours at this site in this strain of rat.

Conclusion by the Notifier

Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 24 month is 31.5 mg/kg bw/day in males and 34.0 mg/kg bw/day in females. It is concluded that glyphosate technical is not carcinogenic in rats. This old study, initiated before the establishment of regulatory testing guidelines, no longer meets current testing guideline criteria due to the low doses employed. Therefore, this study type was repeated by Monsanto with higher doses, in accordance with subsequent regulatory test guidelines.

RMS comment:

It is agreed that this study does not comply to modern standards and should not taken into consideration for evaluation of glyphosate any longer although the NOAEL is agreed with. In particular, the dose levels selected were much too low (in particular to assess carcinogenicity) and there were serious reporting deficiencies.

2nd study: [REDACTED], 1990

Reference:

IIA, 5.5.2/06

Report:

[REDACTED] (1990)

Chronic study of glyphosate administered in feed to Albino rats

Data owner: Monsanto

Project No.: ML-87-148

Date: 1990-09-26

not published, TOX9300244

Guidelines:

US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982); in general accordance with OECD 453

Deviations: (From OECD 453: only 10 rats/sex for interim sacrifice; overall survival at termination was below 50%)
GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 1987-08-05 – 1989-08-10

Materials and methods

Test material:

Identification: Glyphosate

Description: White solid

Lot/Batch #: XLH-264

Purity: 96.5 %

Stability of test compound: Guaranteed for the study period. Confirmed by analysis.

Vehicle and/
or positive control: Diet

Test animals:

Species: Albino Rat

Strain: Sprague-Dawley (CD)

Source:

Age: Approx. 8 weeks (at start of study)

Sex: Males and females

Weight at dosing: Males: approx. 284 g; females: approx. 221 g

Acclimation period: 29 days

Diet/Food: Purina Mills certified Rodent Chow #5002 (Purina Mills), *ad libitum*

Water: Mains drinking water, *ad libitum*

Housing: In stainless steel cages with wire mesh bottoms suspended over paper bedding

Environmental conditions: Animal housing & husbandry were in accordance with the provisions of 'Guide to the Care and Use of Laboratory Animal'; USPHS-NIH Publ. No. 85-23
 Temperature: 17.8 – 21.1 °C
 Humidity: 40 – 70%
 Air changes: not specified
 12 hours light/dark cycle

In life dates: 1987-08-05 to 1989-08-10

Animal assignment and treatment:

In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 2,000, 8000 and 20000 ppm glyphosate (equivalent to mean achieved dose levels of 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females) for 24 months.

A further ten animals per sex were added to each group and were designated for interim kill after 12 month to study chronic toxicity and non-neoplastic histopathological changes.

Test diets were prepared in approximately weekly intervals by mixing a known amount of the test substance with basal diet. The stability of the dietary formulations were determined by analysis of samples of the low- and high-dose levels after storage at room temperature for 7 and 14 days, and frozen after storage for 35 days. The homogeneity of the test substance in the diet was determined for the low- and high-dose level preparations in the first and 88th week of the study. Analyses for achieved concentrations were done for all dose levels for the first six weeks, and for at least one dose level in weekly intervals thereafter. The stability of the neat test substance was verified by analysis before the start of the study, during month 8, 14 and 21, and after termination.

Clinical observations

All rats were examined for mortality and clinical signs of toxicity twice daily. Detailed clinical observations were conducted weekly. An ophthalmic examination was done in all animals before the start of the study, and prior to termination.

Body weight

Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13 and every four weeks thereafter until termination.

Food consumption and compound intake

Food consumption was recorded at weekly intervals for the first 13 weeks, and every fourth week thereafter.

Haematology and clinical chemistry

Blood was collected from 10 fasted animals per sex and group at Months 6, 12, 18, and at termination. The following parameters were measured: haematocrit, haemoglobin, total erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, total bilirubin, direct bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis

Individual urine samples were collected from the same animals as those used for haematology analyses at Month 6, 12, 18 and prior to termination. Sampling was done over a period of about 18-hours via metabolism trays. The following parameters were determined: appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, urobilinogen and blood. In case that blood and / or protein in excess of the control urine samples were found, the sediment was examined for the presence of bacteria, epithelial cells, erythrocytes, leukocytes, casts or abnormal crystals.

Sacrifice and pathology

A gross necropsy was conducted on all surviving animals at scheduled sacrifice after 12 and 24 months. The following organ weights were determined: brain, kidneys, liver, and testes with epididymides.

Tissue samples were taken from the following organs and subjected to a histopathological examination: adrenals, aorta, bone & bone marrow, brain, caecum, colon, duodenum, eyes, gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung (with main stem bronchi), lymph nodes (mesenteric and submandibular), muscle, nasal turbinates, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, sciatic nerve,

seminal vesicles, skin (with mammary tissue), spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary salivary gland, testes with epididymis, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (corpus and cervix).

Statistics

Dunnett's Multiple Comparison Test (two-tailed) was used for body weights, cumulative body weight changes, food consumption, absolute leukocyte counts, reticulocyte counts, urine pH, urine specific gravity and clinical chemistry data obtained at Months 6, 12 and 18. Fisher's exact test (one-tailed) was used for incidence of selected ocular lesions, as well as in combination with Bonferroni inequality procedure for incidences of non-neoplastic (at $p \leq 0.01$) and neoplastic lesions (at $p \leq 0.01$ and ≤ 0.05). EHL decision tree analysis was used for evaluation of terminal haematology, clinical chemistry, body weight, absolute and relative organ weight data and organ to brain weight ratios. Depending on the results either parametric (Dunnett's Test and linear regression) or nonparametric (Kruskal-Wallis, Jonckheere's and / or Mann-Whitney Tests) were applied. Mortality data were analysed by SAS lifetable procedure, and Peto Analysis was used for evaluation of histopathological data.

Results and discussion

Analysis of dose formulations

The stability analyses proved the neat test substance to be stable throughout the study period. The stability and homogeneity of glyphosate in diet at concentrations of 2000 and 20000 ppm was satisfactory. The mean achieved concentrations of glyphosate in each dietary preparation were 95 % of the nominal concentration.

Mortality

There were no statistically significant differences in the group survival rates. The percentage of survival in each of the dose groups are summarised below.

Table B.6.5-27: Percentage survival at termination after 24-month dietary exposure to glyphosate

	Dose group (ppm)			
Sex	0	2000	8000	20000
Male	29	38	34	34
Female	44	44	34	36

Clinical observations

There were no treatment-related clinical sings noted except the ophthalmological findings (see below).

Body weight

There were no effects on body weight noted in males of any dose group. In high-dose females body weights were statistically significant reduced from Week 7 through approximately the 20th month. During this time, absolute body weights gradually decreased to 14 % below the control value. The maximum difference in body weights was observed at 20th month. At this time-point the cumulative body weight gain in high-dose females was 23 % lower as compared to controls

There were no treatment-related effects in females fed 2000 or 8000 ppm glyphosate.

Food consumption and compound intake

There were no statistically significant decreases in food consumption in any group of either sex during the study period. However, significant increased food consumption was noted frequently in high-dose males, and on some occasions in low-dose males. The group mean achieved doses are summarised below.

Table B.6.5-28: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0	0	0
2 (low)	2000	89	113
3 (mid)	8000	362	457
4 (high)	20000	940	1183

Ophthalmoscopy

There were no treatment-related ocular effects observed in females of any dose group, as well as of males of the low-, and mid-dose group. In high-dose males a statistically increased incidence ($p \leq 0.05$) of cataractous lens changes were observed at the ophthalmic examination prior to termination. However, the observed incidence of 25% was within the historical control range of 0-33 %. A second independent ophthalmic examination also performed prior to termination confirmed a statistically significant increase ($p \leq 0.05$) in the incidence of cataractous lens changes in high-dose males (1/14 (control) compared to 8/19 (high dose)). The results are summarised in the table below.

Table B.6.5-29: Incidences of cataract and lens fibre degeneration in males observed during ophthalmic examinations

	Dose group (ppm in diet)*			
	0	2000	8000	20000
1 st examination	0/15	1/22	3/18	5/20**
2 nd examination	0/14	2/22	3/17	8/19**

* number of rats affected / number of rats examined

** statistically significant from control ($p \leq 0.05$)

The histopathological examination confirmed a slightly, but not statistically, increased incidence of degenerative lens changes (i.e. cataract and/or lens fibre degeneration) in high-dose males (see Table B.6.5-30 below).

Table B.6.5-30: Histopathological confirmed incidences of cataract and lens fibre degeneration in males

	Dose group (ppm in diet)*			
	0	2000	8000	20000
Terminal sacrifice	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

* Number of rats affected / number of rats examined

Due to the small number of rats examined ophthalmologically and affected at termination, the results are difficult to interpret. Nevertheless, the occurrence of degenerative lens changes in high-dose males appears to be exacerbated by treatment.

Haematology and clinical chemistry

Haematology and clinical chemistry evaluations noted various changes in the examined parameters. However, the changes were not consistently noted at more than one time point, were within historical control ranges, small in magnitude, and/or did not occur in a dose-related manner. Therefore, they were considered to be either unrelated to treatment or toxicologically insignificant.

The statistically increased alkaline phosphatase level observed in high-dose females at termination was mostly due to an extremely high value for one animal. However, this finding is in line with observation made in other long-term studies in rats.

Urinanalysis

Urine specific gravity was statistically significant increased at the Month 6 examination. The observed statistically significant decreased urinary pH at 6, 18 and 24 months might be related to the renal excretion of glyphosate, which is an acid.

Necropsy

Gross pathology

There were no treatment-related gross pathological findings observed at necropsy.

Organ weights

At interim kill after 12 months relative liver weights were slightly, but statistically significant increased in high-dose males. At terminal sacrifice absolute liver weights, as well as liver to brain weight ratios were also statistically increased in high-dose males. There were no other significant and dose-related effects on organ weights.

Histopathology

Non-neoplastic lesions

Apart from the eye findings mentioned above histopathological examination showed only one other lesion that reached statistical significance. This was an increased incidence of inflammation of the stomach squamous mucosa in females fed 8,000 ppm glyphosate (see Table B.6.5-31).

Table B.6.5-31: Incidence of inflammation and hyperplasia of the stomach squamous mucosa

		Dose group (ppm in diet)*			
		0	2000	8000	20000
Males	Inflammation	2/58	3/58	5/59	7/59
	Hyperplasia	3/58	3/58	4/59	7/59
Females	Inflammation	0/59	3/60	9/60**	6/59
	Hyperplasia	2/59	3/60	7/60	6/59

* Number of rats affected / number of rats examined

** statistically significant at $p \leq 0.01$ (Fisher exact test with Bonferroni inequality)

Although the incidence of this lesion in mid-dose females (15 %) was slightly outside the historical control range (0 – 13.3 %) for the laboratory, there was no dose-related trend across all groups of females, and there was also no significance difference in male rats. Therefore, this finding is considered to be incidental and not related to treatment with glyphosate.

Neoplastic lesions

The only statistically significant difference in neoplastic lesions was an increased incidence of pancreatic islet cell adenomas observed in low-dose males (see Table B.6.5-32). The incidence (14%) in low-dose males was outside the historical control range (1.8 – 8.5 %) for this laboratory, but was in the historical control range (≥ 17 %) observed in reports from other laboratories. In addition, there was no dose-related trend for this finding in the male groups, as indicated by the lack of statistical significance in the Peto trend test. Due to the lack of a dose-related proliferative effect (hyperplasia) and or progression (carcinoma) of this lesion, and as such effects were not observed in females, this finding was not considered to be treatment-related.

Table B.6.5-32: Incidence of pancreatic islet cell findings

Finding	Sex	Dose group (ppm in diet)*			
		0	2000	8000	20000
Hyperplasia	Males	2/58	0/57	4/60	2/59
	Females	4/60	1/60	1/60	0/59
Adenoma	Males	1/58	8/57**	5/60	7/59
	Females	5/60	1/60	4/60	0/59
Carcinoma	Males	1/58	0/57	0/60	0/59
	Females	0/60	0/60	0/60	0/59

* Number of rats affected / number of rats examined

** Statistically significant at $p \leq 0.01$ (Fisher exact test with Bonferroni inequality)

Conclusion by the Notifiers

Based on the study results the NOAEL in rats after chronic exposure to glyphosate for 24 month is 8000 ppm (corresponding to 362 mg/kg bw/day in males and 457 mg/kg bw/day in females). It is concluded that glyphosate is not carcinogenic in rats.

RMS comment:

The study is considered acceptable. However, based on stomach mucosal irritation (although not strictly dose-related) at the two upper dose levels, a NOAEL of 2000 ppm (89 mg/kg bw/day) was established. Further toxic effects on body weight, liver and eyes (cataracts) were apparently confined to the top dose level.

3d study: [REDACTED], 1993

Reference: IIA, 5.5.2/04

Report:

[REDACTED]
(1993)

Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks)

[REDACTED]
Data owner: Cheminova

Study No.: 438623; Report No.: 7867

Date: 1993-04-07

GLP:

not published, TOX9750499

Dates of experimental work: 1990-02-16 to 1992-03-09

Test material:

In life dates: 1990-02-16 to 1992-03-09

Animal assignment and treatment:

In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 10, 100, 300 or 1000 mg/kg bw/day glyphosate technical. An additional five groups with 35 rats per sex receiving daily dietary doses of 0, 10, 100, 300, or 1000 mg/kg bw/day were included for the toxicity study. Fifteen rats per sex and per dose of the toxicity study were scheduled for interim sacrifice after 12 months. The dose levels were selected based on the results of a 13-week dietary toxicity study in rats.

Test diets were prepared once per week for the first 13 weeks and at least once every two weeks thereafter by direct admixture of the test substance to the plain diet and mixing for 20 minutes.

Analyses for achieved concentrations of the test substance in the diet were conducted from formulated diets at approximately fortnight intervals for the first 12 weeks and in intervals of 2 month thereafter.

The stability and homogeneity of the test substance in the diet was determined prior to the start of the study.

Clinical observations

A check for mortality was made twice daily on all animals throughout the study. In addition, all animals were examined for clinical signs during each day. A detailed clinical examination and check for palpable masses were done once each week on every animal. An ophthalmoscopic examination was conducted on 20 rats per sex of each group of the oncogenicity study before the start of the study and on 20 rats per sex of the control and high-dose group of the oncogenicity study at weeks 24 and 50. In addition, an ophthalmoscopic examination was conducted on all control and high-dose rats of the oncogenicity and toxicity study at week 102.

Body weight

Individual body weights were recorded for each animal before dosing, at weekly intervals until the end of week 13 and approximately every 4 weeks thereafter until termination.

Food and water consumption and compound intake

Food consumption was recorded once weekly for each cage group starting one week before treatment until Week 13 and subsequently every 4 weeks until termination. Water consumption was monitored by visual inspection throughout the study period.

Achieved dosages were calculated from nominal dietary concentration, taking into account actual food consumption and body weight data.

Haematology and clinical chemistry

Individual blood samples for haematology and clinical chemistry evaluations were collected from the orbital sinus of 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point. Individual blood smears for differential blood counts were taken from the tail vein after approximately 52, 78, and 103 weeks of dosing from all surviving animals of the oncogenicity study.

Haematology

The following parameters were measured: Haemoglobin, haematocrit, total erythrocyte count total leukocyte count, differential leukocyte count, platelets, and clotting time. Absolute indices were calculated.

Differential blood counts were evaluated with blood smear samples from all control and high-dose animals of the oncogenicity study at weeks 53 and 79. In addition, differential blood cell counts were evaluated on all surviving animals of the oncogenicity study at week 104.

Blood chemistry

The following parameters were measured: Total proteins, albumin, albumin-globulin ratio, ALT, AST, ALP, blood urea nitrogen, blood glucose, sodium, potassium, chloride,

cholesterol, creatinine, calcium, phosphate, total bilirubin, plasma cholinesterase, creatinine phosphokinase and red blood cell cholinesterase.

Brain cholinesterase activity determination

Brain cholinesterase activity was determined from 10 rats per sex from each dose group at the week 52 and 104 necropsies. Approximately 0.5 g of brain was removed at the week 52 and 104 necropsies and stored at -20°C until analysis.

Urinalysis

Individual urine samples were collected from 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point. Samples were collected over a period of 4 hours of food and water deprivation in metabolism cages. The following measurements were made: volume, specific gravity, pH, urobilinogen, bilirubin, blood pigments, protein, glucose, ketones, microscopy of sediments.

Sacrifice and pathology

At interim kill after 52 weeks 15 rats per sex from each toxicity study group were sacrificed and necropsied. All remaining toxicity study and surviving oncogenicity study animals were killed and necropsied after 104 weeks. All pre-terminally dead and moribund sacrificed rats were also necropsied.

The following organs were weighed from all interim kill animals of the toxicity study and from 10 rats per sex per group of the oncogenicity study: adrenals, brain, heart, kidneys, liver, lungs, ovaries (with fallopian tubes), parotid salivary glands, pituitary, prostate, sublingual and submaxillary salivary glands (weighed together), spleen, testes including epididymides, thymus and uterus.

The following organs were collected: adrenals, aortic arch, any abnormal tissue, bladder, bone and bone marrow (sternum and rib), brain, ears, eyes, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric and submandibular), muscle (thigh), nasal cavity (oncogenicity study only), oesophagus, optic nerve, ovaries (with fallopian tubes), pancreas, parotid salivary glands, pituitary, prostate, sciatic nerve, seminal vesicles, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach (glandular and non-glandular), sublingual salivary glands, submaxillary salivary glands, testes with epididymides, thymus, thyroid/parathyroid, tongue, trachea, uterus and vagina.

A detailed histopathological examination was performed on all tissues collected from the control and high-dose animals at interim kill, all oncogenicity study animals, and all animals that died or were killed in extremis. In addition, a histopathological examination of the liver, kidneys and lungs was performed on all other toxicity study animals at interim kill and all oncogenicity study animals. Furthermore, the salivary glands of all low- and mid-dose animals at interim kill and the oncogenicity study were examined.

Statistics

Haematology, clinical chemistry, organ weight and body weight data were analysed for homogeneity of variance using the F-max test. If the group variances appeared homogeneous a parametric ANOVA was used and pair wise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variances were heterogeneous log or square root, transformations were used. If the variances remained heterogeneous a non-parametric test (e.g., Kruskal-Wallis ANOVA) was used. Organ weights were also analysed conditional on body weight (i.e., ANOVA). Differences in survival between the control and test substance

groups from the oncogenicity study were assessed graphically using Kaplan-Meier plots and tested formally using the Gehan-Wilcoxon test. Because no notable survival differences were evident, histological lesion incidences were analysed using Fisher Exact test.

Results and discussion

Analysis of dose formulations

Analyses for achieved concentrations showed that the diet preparations of all dose groups were within an acceptable degree of accuracy ($\pm 10\%$).

Mortality

There were 336 pre-terminal deaths throughout the study. There was no evidence to suggest that any of these deaths were treatment related. There were also no significant treatment-related effects on the survival times in the oncogenicity study.

The numbers of pre-terminal deaths are summarised in Table B.6.5-33 below.

Table B.6.5-33: Cumulated mortalities after 104-week dietary exposure to glyphosate technical

	Dose group (mg/kg bw/day)*				
Sex	0	10	100	300	1000
Male	27/85	32/85	25/85	26/85	26/85
Female	42/85	41/85	42/85	40/85	35/85

*number of dead / total number

Clinical observations

The only notable clinical sign was pale faeces, from weeks 16-104, the majority or all the cages of animals (males and females) in the 300 and 1000 mg/kg /day dose groups had pale faeces. However, this clinical sign was not considered to be toxicologically significant. There were no other notable clinical signs considered to be treatment related.

Ophthalmoscopy examinations demonstrated no inter-group differences.

Body weight

The high-dose group males and females had statistically significant reductions in body weight throughout the study. Reductions started at week one of dosing and were still apparent at week 104. The high-dose group males displayed the greatest reduction in body weights and body weight gains. The mean body weight gain data are summarised in Table B.6.5-34 below.

Table B.6.5-34: Body weight development (mean values) after 52 and 104-week dietary exposure to glyphosate technical – oncogenicity study

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Weight gain (g) (0-52 weeks)	514	265	498	285	523	270	500	274	450	243
% of control	--	--	97	108	102	102	97	103	88	92
Weight gain (g) 0-104 weeks	635	376	609	445	644	391	623	405	549	333
% of control	--	--	96	118	101	104	98	108	86	89

Food and water consumption and compound intake

There were no treatment-related effects on food and water consumption for either sex noted during the study.

The overall group mean achieved doses are summarised in Table B.6.5-35 below.

Table B.6.5-35: Group mean achieved dose levels – oncogenicity study

Dose group	Nominal dose (mg/kg bw/day)	Mean achieved dose level (mg/kg bw/day)		Mean achieved dose level (% of nominal)	
		Males	Females	Males	Females
1 (control)	0	--	--	--	--
2 (low)	10	10	10	100	100
3 (mid I)	100	101	103	101	103
4 (mid II)	300	306	311	102	104
5 (high)	1000	1007	1018	101	102

Over the entire study duration the mean achieved dosages in all groups were close to the nominal.

Haematology and clinical chemistry

Haematology

Haemoglobin, haematocrit and mean corpuscular haemoglobin were occasionally increased in 100 and 1000 mg/kg bw/day dose group males. Haemoglobin was also increased in males from the 300 mg/kg bw/day dose group and females from the 1000 mg/kg bw/day group. Females of the 1000 mg/kg bw/day dose group also had increased levels of mean corpuscular haemoglobin.

The haematological changes were not considered to be treatment related due to the lack of a clear dose-response relationship. In addition, the differences observed were rather small and no consistent trend became obvious throughout the study. In the absence of any histopathological change these small increases are not considered to be of toxicological significance (see Table B.6.5-36).

Clinical chemistry

Clinical chemistry measurements showed significant increased alkaline phosphatase levels in males at 1000 mg/kg bw/day and in females at 100, 300 and 1000 mg/kg bw/day. Although the increases were of small magnitude they were consistent and might be treatment-related. However, in the absence of any histopathological changes these small changes are not considered to be of toxicological significance (see Table B.6.5-37). All other changes in clinical chemistry parameters were not considered to be treatment-related.

Table B.6.5-36: Haematology findings (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Haemoglobin (g/dL)										
Week 14/15	15.6	15.5	15.8	15.0*	16.2	15.0*	16.2	15.5	16.2	15.9
Week 25/26	15.3	15.2	15.5	14.9	16.1***	14.9	15.9*	15.4	16.4***	15.6
Week 51/52	15.3	14.7	15.5	14.6	15.9	14.5	15.4	14.7	15.6	15.3*
Week 78/79	15.1	14.1	14.3	13.8	15.7	14.4	14.6	14.4	15.4	15.1
Week 102/103	14.0	12.1	13.1	13.6	14.3	13.1	13.8	13.3	14.6	12.9
Haematocrit (L/L)										
Week 14/15	0.397	0.396	0.405	0.386	0.406	0.387	0.407	0.395	0.411	0.407
Week 25/26	0.388	0.392	0.389	0.389	0.409**	0.384	0.399	0.398	0.409**	0.403
Week 51/52	0.406	0.394	0.415	0.388	0.415	0.386	0.410	0.392	0.414	0.408
Week 78/79	0.405	0.382	0.386	0.375	0.415	0.382	0.392	0.387	0.411	0.406
Week 102/103	0.392	0.343	0.365	0.381	0.394	0.367	0.387	0.369	0.401	0.363
MCH (pg)										
Week 14/15	21.3	22.6	21.1	22.5	21.7	22.4	21.9	22.4	21.8	22.8
Week 25/26	21.2	22.4	21.4	22.4	21.9	22.4	21.9	22.2	22.0	22.8
Week 51/52	20.2	22.1	20.1	22.3	21.1*	22.4	20.8	22.2	20.9*	22.7
Week 78/79	20.1	22.3	19.7	22.4	20.8*	22.4	20.6	23.0	20.9*	23.1**
Week 102/103	20.4	22.3	20.1	22.3	20.1	22.0	20.9	22.6	20.6	22.7
WBC (x 10 ⁹ /L)										
Week 14/15	14.0	12.0	14.5	13.3	13.4	12.0	13.7	11.1	14.2	12.0
Week 25/26	13.4	8.8	13.2	10.3	11.8	9.9	12.2	8.9	12.7	10.5
Week 51/52	12.8	7.9	13.7	9.1	11.7	7.7	12.9	7.4	12.4	8.8
Week 78/79	12.4	7.7	13.6	7.3	10.9	8.1	13.6	6.8	10.6	7.0
Week 102/103	10.5	10.1	12.2	7.1*	10.3	6.4**	11.6	7.3*	9.5	8.4
Lymphocytes (x 10 ⁹ /L)										
Week 14/15	11.7	10.8	12.6	11.9	12.0	10.9	11.8	9.2	12.2	10.7
Week 25/26	10.7	7.1	10.8	8.2	9.6	8.1	10.1	7.4	10.3	8.6
Week 51/52	10.9	6.5	11.0	7.4	9.7	6.6	10.8	6.0	10.3	7.5
Week 78/79	10.0	5.7	10.3	5.6	8.7	6.4	10.1	4.8	8.5	5.6
Week 102/103	7.6	5.7	8.0	4.8	7.3	4.3**	7.8	4.7*	6.7	5.6

* p < 0.05; ** p < 0.01; *** p < 0.001

Table B.6.5-37: Clinical chemistry findings (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
ALP (IU/L)										
Week 14	287	182	329	158	320	213	334	223	461***	244*
Week 25	251	148	272	152	267	201*	306	227**	367**	225**
Week 51	308	144	293	143	310	190*	353	195*	403	221**
Week 78	258	124	286	139	284	172	351*	207**	414***	186*
Week 102	212	190	265	161	287*	193	267	228	365***	286*

* p < 0.05; ** p < 0.01; *** p < 0.001

Urine analysis

Urinary pH was slightly reduced in males at 1000 mg/kg bw/day. This change was consistent with that found in a previously conducted 13-week toxicity study with glyphosate.

Necropsy

Gross pathology

There were no treatment-related macroscopic findings observed at the interim and terminal kill necropsies.

Organ weights

At the interim kill (week 52) absolute liver weights were reduced in males and females at doses of 100 mg/kg bw/day and above. However, for males this finding was not confirmed by the sensitive means of covariance analysis, i.e., with correction for final body weight. Absolute adrenal weights were reduced in males at 300 and 1000 mg/kg bw/day. However, this finding was also not confirmed by the sensitive means of covariance analysis, i.e., with correction for final body weight.

At the terminal kill (week 104) no statistical significant decrease in liver and adrenal weights was noted in any dose group. Absolute kidney weight was reduced in males at 100 and 1000 mg/kg bw/day after 104 weeks, but a clear dose relationship was lacking.

At 52 weeks parotid salivary gland weight was increased in males at 100, 300 and 1000 mg/kg bw/day. Combined sublingual and submaxillary gland weight was increased in high-dose males and females. However, salivary gland weights were not affected at week 104 at any dose level.

Histopathology

The most notable histological finding was seen in the salivary glands where cellular alteration was seen in submaxillary and parotid salivary glands in males and females at 300 and 1000 mg/kg bw/day at week 52, and in both sexes at 100, 300 and 1000 mg/kg bw/day at week 104. These changes followed a dose-related pattern and are considered to be treatment related; however, these cellular alterations are similar to those seen occasionally in other subchronic or long-term dietary studies and are considered an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and are of no adverse consequence.

Another histopathological finding was a decreased incidence of nephropathy in males at 100, 300 and 1000 mg/kg bw/day at interim kill. This finding was also noted in high-dose males at 104 weeks, but with reduced severity. Nephropathy is a common finding in old rats and as the incidence is decreased this finding is not considered as toxicologically significant.

In addition, the decreased incidence of urothelial hyperplasia in high-dose females at week 52 and 104, as well as in females at 300 mg/kg bw/day at week 104, is also not considered to be of toxicological significance.

Neoplastic changes

Neoplastic lesions were seen in all dose groups, however there was no dose relationship in the incidence of any individual tumour or in the incidence of animals with tumours.

It is concluded that the test compound at dose levels up to and including 1000 mg/kg bw/day produced no carcinogenic effect.

Conclusion by the Notifiers

Based on the study results and the lack of toxicological significance of the salivary gland findings, as well as a slight increase of plasma alkaline phosphatase observed at 300 mg/kg bw/day, the NOAEL in rats after chronic exposure to glyphosate technical for 104 weeks is considered to be 300 mg/kg bw/day. It is concluded that glyphosate technical is not carcinogenic in rats.

RMS comment:

The study is considered acceptable. However, the NOAEL is rather 100 mg/kg bw/day because histological alterations of the salivary glands were clearly more pronounced at the two upper dose levels and were accompanied by a clear increase in parotid gland weight after 1 year. The AP increase at these dose levels were in parallel to a liver weight at least after one year. Therefore, 300 mg/kg bw/day is considered the LOAEL. Effects at the NOAEL were still be seen but minor in nature. This evaluation is in line with the most recent one of WHO/FAO (JMPR, 2004, ASB2008-6266).

4th study: [REDACTED], 1996

Reference: IIA, 5.5.2/01

Report: [REDACTED] (1996)

Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats

Data owner: Feinchemie Schwebda GmbH

Study No.: 886.C.C.R

Date: 1996-07-18

GLP:

not published, TOX9651587

Guidelines: OECD 453 (1981)

Deviations: Individual animals exceed the 20% range in body weight; organ weights were not determined for all animals; weights of heart, spleen and (para)thyroids are missing

GLP: yes

Acceptability: See RMS comment

Dates of experimental work: 1992-03-04 - 1994-03-04

Materials and methods

Test material:

Identification: Glyphosate technical

Description: White odourless crystals

Lot/Batch #: 60; 046

Purity: 96.8 %; 96.8 %

Stability of test compound: More than two years at ambient temperature

Vehicle: Diet

Test animals:

Species:	Rat
Strain:	Wistar
Source:	
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	Males: 90 – 179 g, females: 80 – 151 g
Acclimation period:	At least one week
Diet/Food:	Standard "Gold Mohur" (M/S Lipton India Ltd, India), <i>ad libitum</i>
Water:	Deep bore well water treated with charcoal filter and UV rays, <i>ad libitum</i>
Housing:	Initially in groups of five per sex in polypropylene cages and in groups of three from Week 12 onwards.
Environmental conditions:	Temperature: 19 -25 °C Humidity: 40 - 70% Air changes: not reported 12 hours light/dark cycle

In life dates: 1992-03-04 to 1994-03-04

Animal assignment and treatment:

In a combined chronic toxicity and carcinogenicity study groups of 50 Wistar rats per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 7.4, 73.9 and 740.6 mg/kg bw/day for 24 months respectively) glyphosate technical. In addition one vehicle control with ten rats per sex and one high dose group with 20 rats per sex were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes.

Test diets were prepared fortnightly by mixing a known amount of the test substance with a small amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 20 minutes.

The stability of the test substance in food was determined in an in-house stability study at 2000 and 20000 ppm.

Observations

Veterinary examination was made before and after grouping and at the end of each month of experimental schedule. Rats were examined for toxic signs and pre-terminal deaths once a day. Ophthalmic examination was done at the start of the study and at termination.

Body weight

Individual body weights were recorded before dosing, at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake

Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination.

Haematology and clinical chemistry

Haematology

Individual blood samples were collected from 20 rats/sex/group at 3, 6, 12, 18 and 24 months. The following parameters were measured: Haemoglobin, haematocrit, erythrocyte count, clotting time and total leukocyte count and differential leukocyte count.

Blood chemistry

At the scheduled intervals of 6, 12, 18 and 24 months, blood collected from 10 rats/sex/group was subjected to clinical chemistry analysis. The following parameters were measured: Total proteins, albumin, ALT, AST, GGT, ALP, blood urea nitrogen and blood glucose.

Urinalysis

Individual urine samples were collected from 10 rats/sex/group at 3, 6, 12, 18 and 24 months. The following measurements were made: Volume, appearance, pH, nitrite, urobilinogen, bilirubin, erythrocytes, protein, glucose, ketones, microscopy of sediments.

Sacrifice and pathology

Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups.

The following organ weights were determined from 10 rats per sex per group: adrenals, brain, gonads, kidneys and liver.

Tissue samples were taken from the following organs: adrenals, aorta (main group animals), bone & bone marrow (sternum and femur incl. joint), brain, caecum, colon, duodenum, epididymides (main group animals), eyes (with optic nerve), heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric, mandibular and mediastinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, tumour/mass, urinary bladder and uterus.

A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.

Statistics

Using specific computer programs, body weight, net body weight gain, food consumption, haematology, clinical chemistry and organ weight data of different groups were compared by Bartlett's test for homogeneity of intra group variances. When the variances proved to be heterogeneous, the data were transformed using appropriate transformation. The data with homogeneous intra group variances were subjected to one-way analysis of variance (ANOVA - Snedecor and Cochran). When 'F' value was significant, Dunnett's pair wise comparison (Scheffe) of means of treated groups with control mean was done individually.

Net food intake (g/kg bw/d) and test compound intake (mg/kg bw/d) was calculated for the whole study period using calculated means and food intake was statistically analysed by the procedure given above. Incidence of gross, histopathological changes of mass(es) and incidence of benign and malignant neoplasia in the treatment groups were statistically compared with control group by Z-test wherever it was applicable/necessary. The incidence

of neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.

When a significant difference to the control was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t' test.

Results and discussion

Analysis of dose formulations

Analyses for achieved concentrations showed that the diet preparations of the control, low, mid- and high dose group were within an acceptable range. The mean achieved concentrations of the test substance of eight batches of the prepared test substance diets were 0.0, 99.1 ± 4.7 , 995.3 ± 36.8 and 9993.1 ± 277.5 ppm, for the control, low, mid and high dose group, respectively.

Mortality

There were no treatment-related deaths observed during the study.

The numbers of pre-terminal deaths in the carcinogenicity study groups are displayed in Table B.6.5-38.

Table B.6.5-38: Cumulated mortalities after 104-week dietary exposure to glyphosate technical

	Dose group (ppm)			
Sex	0	100	1000	10000
Male	30	30	32	21
Female	26	24	17	29

Clinical observations:

There were no treatment-related clinical signs of toxicity observed during the study.

Body weight:

There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

Food consumption and compound intake

There were no treatment-related effects on food consumption for either sex noted during the study. The group mean achieved doses are summarised below.

Table B.6.5-39: Group mean achieved dose levels in the main groups

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)		
		Males	Females	Overall mean
1 (control)	0			
2 (low)	100	6.3	8.6	7.4
3 (mid)	1000	59.4	88.5	73.9
4 (high)	10000	595.2	886.0	740.6

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 7.4, 73.9 and 740.6 mg/kg bw/day for 100, 1000, and 10,000 ppm, respectively.

Haematology and clinical chemistry

The following significant dose related changes of the blood chemistry parameters were seen at the high dose:

decrease in GGT level at 12 months in male rats

decrease in Albumin level at 6 months in female rats

increase in AP (alkaline phosphatase) level at 6 months in female rats

No other dose or treatment related significant changes were observed in haematological, and biochemical parameters. These changes observed were only temporal and were not consistently seen at all sampling periods throughout the study. The dose related changes were also within the range of the historical control data and hence appear to be of no biological significance.

Table B.6.5-40: Statistically significant changes in blood chemistry

Parameters	Timepoint	Dose group (ppm)							
		0		100		1000		10000	
		♂	♀	♂	♀	♂	♀	♂	♀
Albumin (g/dL)	6 month	4.0	3.7	3.9	3.7	4.0	3.7	3.9	3.5*
Alkaline phosphatase (U/L)	6 month	213	133	251	146	227	153	185	235*
GGT (U/L)	12 month	8.3	5.8	8.3	7.7	8.4	6.3	5.1*	5.3

* $p < 0.05$;

Urinalysis

There were no treatment-related findings.

Necropsy

Gross pathology

There were no treatment-related macroscopic findings observed during the study period.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

None of the significant microscopic changes, both increased and decreased incidences (in liver, spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) observed have shown dose relationship, hence appeared to be incidental and not related to the treatment with the test compound.

Neoplastic changes

The historical data on neoplasm incidence for the test species indicates that the incidences of various tumours observed in the present study are within the range. The types of tumours seen were also comparable to the historical records.

No statistically significant inter group difference between the control and low, mid and high dose treatment groups has been recorded in respect of the number of rats with neoplasms, number of malignant neoplasms and incidence of metastasis either sexwise or for combined sex.

Table B.6.5-41: Summary of neoplastic histopathological findings

	Dose group (ppm)
--	------------------

	Males				Females			
	0	100	1000	10000	0	100	1000	10000
Findings for dead and moribund sacrificed animals								
Cholangiocarcinoma	0/30	2/30	2/32	2/21	1/26	0/23	0/17	0/29
Hepatocellular adenoma	9/30	9/30	6/32	6/21	2/26	8/23	3/17	5/29
Hepatocellular carcinoma	12/30	12/30	9/32	5/21	4/26	4/23	2/17	5/29
Intrahepatic bile duct adenoma	1/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Histiocytic sarcoma	2/30	0/30	2/32	1/21	1/26	0/23	0/17	0/29
Fibrosarcoma	0/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Findings for animals sacrificed at termination								
Cholangiocarcinoma	1/20	1/20	0/16	1/29	0/24	0/25	0/32	0/21
Hepatocellular adenoma	15/20	13/20	4/20	15/20	16/24	10/25	16/32	8/21
Hepatocellular carcinoma	9/20	16/20	9/16	19/29	6/24	11/25	12/32	4/21
Intrahepatic bile duct adenoma	1/20	0/20	0/16	0/29	6/24	11/25	12/32	4/21
Histiocytic sarcoma	0/20	1/20	1/16	0/29	0/24	1/25	0/32	0/21
Benign mixed intra-hepatic bile duct adenoma	0/20	0/20	1/16	0/29	0/24	0/25	0/32	0/21

Incidentally, the number of benign tumours in the low and mid dose group males and combined sex was lower and higher in the mid dose group females. There was no dose-response relationship and the significances were considered incidental.

The different liver tumours observed in the dead and moribund sacrificed and terminally sacrificed rats included hepatocellular adenoma, intrahepatic bile duct adenomas, cholangiocarcinoma, hepatocellular carcinoma, histiocytic sarcoma, fibrosarcoma and lymphosarcoma. Of these, hepatocellular adenomas and carcinomas occurred more frequently, as often observed in ageing rats. The occurrence of these tumours appeared to be incidental and not compound-related as their frequency of occurrence was not dose dependent. No reasons could be ascribed for the decrease in the number of benign tumours in the low and mid dose group males and for combined sex and for an increase seen in the mid group dose females (see Table B.6.5-41).

From this, it is concluded that the test compound at the doses tested does not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.

Conclusion by the Notifiers

Based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 595 mg/kg bw/day for males, and 886 mg/kg bw/day for females (740 mg/kg bw/day for combined). It is concluded that glyphosate technical is not carcinogenic in rats.

RMS comment:

The study is considered acceptable. However, it should be amended that AP activity was increased in female rats not only at 6-month sampling but also after 12 and 18 months achieving statistical significance for the two upper dose levels (unfortunately not shown in Table B.6.5-6 but in the EU DAR). Because this increase that was observed in other studies with glyphosate, too, was not accompanied by further liver findings, it is not considered adverse. However, there was a slight increase in cataract frequency with 3, 4, 2, and 7 males and 1, 4, 5, and 4 females being affected in the control and the treatment groups at study termination. Since a similar finding was reported by [REDACTED] (1990, TOX9300244), a treatment-related and adverse effect of glyphosate administration cannot be excluded. Accordingly, the NOAEL was set at 1000 ppm, i.e., ca 60 mg/kg bw/day.

The RMS is not aware of any further long-term studies with the active ingredient, apart from an earlier IBT study ([REDACTED] 1974, Z35230) on rats in which only low dose levels were employed with some minor liver effects occurring at the highest dose of 300 ppm after 2 years. This study was mentioned by [REDACTED] (1981, TOX2000-595) but was not submitted for EU evaluation.

B.6.5.2 Carcinogenicity in the mouse

The carcinogenicity studies by [REDACTED] (1983, TOX9552381) and by [REDACTED] (1993, TOX9552382) are reported in detail in the original DAR (1998, ASB2010-10302). They have been both re-evaluated by the RMS and were confirmed as acceptable. Core information, NOAELs/LOAELs and the outcome of re-evaluation are mentioned in Vol. 1 (2.6.5).

Two more long-term studies in mice had been submitted for previous EU evaluation ([REDACTED] 1988, TOX9551831; [REDACTED], 1982/1992, TOX9650154) but were considered not acceptable for reliable assessment of carcinogenicity. Thus, they were not taken into consideration for this re-evaluation. A brief description may be found in the original DAR (1998, ASB2010-10302).

In the following, descriptions of the new studies (i.e., those not previously used for EU evaluation) are given. Reporting of these studies is based on the GTF dossier. Comments by the RMS may be found below the individual studies. A summary view on carcinogenicity studies in the mouse is presented in Vol. 1 (2.6.5).

1st new long-term study in mice ([REDACTED] 2001)

Reference: KIIA 5.5.3
Report: [REDACTED] (2001)
 Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice
 [REDACTED]
 Data owner: Feinchemie Schwebda GmbH
 Study No.: Toxi: 1559.CARCI-M
 Date: 2001-10-10
 not published, ASB2012-11491
Guidelines: OECD 451 (1981)
Deviations: None
GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 1997-12-18 - 1999-06-29

Materials and methods

Test material:	Glyphosate technical
Identification:	Glyphosate
Description:	Solid white, odourless crystals
Lot/Batch #:	01/06/97
Purity:	> 95 % (w/w)
Stability of test compound:	Expiry: December 1999
Vehicle and/ or positive control:	Diet
Test animals:	
Species:	Mouse
Strain:	Swiss albino, HsdOla: MF1
Source:	
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	Males: 25 – 47 g, females: 21 – 26 g
Acclimation period:	5 days
Diet/Food:	Ssniff rat/mouse powder food maintenance meal – low in germs (M/s Ssniff Spezialdiäten, D-59494 Soest, Germany), <i>ad libitum</i>
Water:	Well water passed through activated charcoal filter and exposed to UV rays, <i>ad libitum</i>
Housing:	In groups of five per sex in polypropylene mouse cages with stainless steel top grill and staem sterilized clean paddy husk bedding.
Environmental conditions:	Temperature: 19 - 25 °C Humidity: 30 - 70 % Air changes: 12 - 15/hour 12 hours light/dark cycle

In life dates: 1997-12-23 to 1999-06-29

Animal assignment and treatment:

In a carcinogenicity feeding study groups of 50 Swiss albino mice per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 14.5, 149.7 and 1453 mg/kg bw/day for males, and 0, 15.0, 151.2 and 1466.8 mg/kg bw/day for females) glyphosate technical in diet for 18 month. The dose levels were chosen based on results of a 50-day pre-study in mice. Test diets were prepared prior to start of treatment and then in intervals ranging from 10 to 23 days. Diets were prepared in quantities of 10, 12 or 15 kg. For preparation of 12 kg diet mixtures 1.2 g, 12 g and 120 g for the low-, mid- and high-dose group, respectively, of the test substance was mixed with approximately with 0.5 kg basal diet and blended for 3 minutes. This pre-mix was then mixed manually with approximately 0.5 kg food and then added in portions to the remaining bulk amount of food (approximately 11.0 kg) and blended in a stainless steel ribbon mixer for 20 minutes.

The homogeneity of the test material in diet was determined at beginning of treatment, and at 12 and 18 month. Analyses for achieved concentration were done at three and six month of

the study. The stability of glyphosate technical in the diet was determined prior to start of the study for the 100 and 10000 ppm dose levels.

Clinical observations

A detailed veterinary examination of all mice was done before and after grouping and monthly thereafter. A check for clinical signs of toxicity, appearance, behaviour, and neurological changes and mortality was made once daily on all mice. For mice with observed tumours a separate record was maintained with details of the tumour development.

Ophthalmological examination

Ophthalmological examinations were performed on all mice prior to start of treatment at 6, 12 and 18 month of the study. Mydriasis was induced before examination by adding 1% Tropicamide solution into the eyes. All other grossly visible eye findings were recorded also at the daily observations.

Body weight

Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake

Food consumption was recorded once weekly for each cage group from week 1 to week 13 and subsequently at weeks 26, 39, 52, 65 and 68. Food efficiency and compound intake was calculated from the recorded food consumption data.

Haematology

Blood smear samples were collected at 9 month and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.

Sacrifice and pathology

All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately or preserved in 10% buffered neutral formalin until necropsy.

All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.

The following organ weights were determined from 10 mice per sex per group: adrenals, kidneys, liver and gall bladder, ovaries, and testes.

Tissue samples were taken from each mice from the following organs and preserved in 10% buffered neutral formalin: adrenals, bone & bone marrow (sternum and femur (incl. joint)), brain (incl. cerebrum, cerebellum pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), heart, jejunum, kidneys, larynx, liver and gall bladder, lungs, lymph nodes (mandibular, mesenteric, and superficial inguinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and all lesions and tumours/masses.

A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of gross lesions and masses from all mice were examined microscopically.

Statistics

Body weight, body weight gain, food consumption and differential leukocyte counts of different groups were compared by Bartlett's test for homogeneity of intra group variances. Heterogeneous data were transformed using log transformation. Data with homogeneous intra group variances were subjected to one-way analysis of variance using ANOVA. When "F" values were significant, Dunnetts pair wise comparison of means of treated groups with control means was done individually.

Incidence of gross lesions and non-neoplastic histopathological changes and incidences of benign and malignant neoplasms in the test substance groups were statistically compared with control group by Z-test where necessary. The incidence of neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis. When a significant difference over the control group was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t-test. All analyses and comparisons were evaluated at the 5 % level and statistically significant differences ($p \leq 0.05$) were indicated.

Results and discussion

Analysis of dose formulations

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss of 8.37 % at the 100 ppm level and 6.99 % at the 10000 ppm level, when stored at room temperature in PE bags inside stainless steel drums.

Analyses for homogeneity at the start and at 12 and 18 month of treatment indicated that the dose preparations were homogeneous. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within $\pm 10\%$ of the nominal concentration for all diet samples. The overall mean achieved concentrations were 94.0 ± 1.66 , 949.5 ± 15.84 and 950.7 ± 142.28 as compared to the nominal concentrations of 100, 1000 and 10000 ppm, respectively.

Mortality

The cumulated pre-terminal deaths (including moribund sacrifice) are summarised in Table B.6.5-42 below.

Table B.6.5-42: Cumulated mortalities after 78-week dietary exposure to glyphosate technical

Sex	Historical control [#]		Dose group (ppm) ^{**}			
	Min- max	Mean \pm SD	0	100	1000	10000
Male	11/50 – 27/50	18 ± 5	22 (6)	20 (6)	22 (8)	27 (8)
Female	12/50 – 20/50	16 ± 3	16 (7)	16 (7)	20 (2)	20 (3)
Combined sex	12/100 – 47/100	17 ± 4	38 (13)	36 (13)	42 (10)	47 (11)

[#] Derived from the control groups of 9 studies performed in the timeframe embracing the study summarised here

^{*} Number of dead animals / total number of animals

^{**} Total number of animals per group = 50

() number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table B.6.5-43: Percentage survival at termination after 18-month dietary exposure to glyphosate technical

	Dose group (ppm)			
Sex	0	100	1000	10000
Male	56	60	56	46
Female	68	68	60	60
Combined	62	64	58	53

The survival percentage was slightly decreased at the high dose level but the decrease did not attain statistical significance.

As can be seen from the historical control data, the mortality in the high-dose group is still within the historical control range even though at the upper end.

Clinical observations

There were no significant treatment-related clinical signs of toxicity observed.

Body weight

There were no significant treatment-related effects on male and female body weight and overall body weight gain during the conduct of study.

In males, incidences of slightly decreased body weights in week 10 at 100 ppm and in months 7 and 8 at 1000 ppm were considered incidental, since no effects on body weights were observed in the high-dose group.

In females, decreased net body weight gain was observed in month 18 at 100 ppm only. Therefore, this finding was also considered as incidental.

Food consumption and compound intake

There were no treatment-related effects on food consumption for either sex noted during the study.

The observed slightly lower food consumption observed in males in week 1 at 100 ppm and in weeks 1 and 7 at 10000 ppm was considered incidental since the changes were minimal and the effects was not consistent during the remaining parts of the study period.

In females, lower food consumptions were observed in week 2 for all dose levels and in week 26 at 10000 ppm. Higher food consumption occurred in week 11 at 100 ppm and in weeks 3 and 4 at 10000 ppm. These findings were also considered incidental since the changes were minimal and food consumption during the remaining parts of the study was comparable with the control group.

The calculated mean daily test substance intake is summarised in Table B.6.5-44 below.

Table B.6.5-44: Group mean daily compound intake levels

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	100	14.5	15.0	14.7
3 (mid)	1000	149.7	151.2	150.5
4 (high)	10000	1453.8	1466.8	1460.3

* based on actual food intake and body weight data

Haematology

Differential leukocyte counts at 9 and 18 month

There were no significance treatment-related changes in the white blood cell counts for either sex at both 9 and 18 month. Slightly higher neutrophile counts and slightly lower lymphocyte counts in high dose males at 9 month were within the historical control ranges. The slightly higher eosinophil counts, higher neutrophil and monocyte counts, as well as slightly lower lymphocyte counts observed in high dose females at 18 month were comparable with historical control values and therefore considered incidental.

Differential leukocyte counts of moribund sacrificed mice

Although the differential leukocyte count data were not statistically analysed, they appeared to be within the range of biological variation.

Ophthalmological examination

There were no treatment-related findings observed at the ophthalmological examinations performed at 6, 12 and 18 month of treatment.

Gross pathology

There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.

In animals found dead or sacrificed moribund across control and all dose levels the incidence of enlargement of superficial inguinal lymph nodes and thymus in mid dose females and in the high dose for combined sexes was statistically significantly increased. These enlargements were associated with neoplasms of the hemolymphoreticular system. Other changes included enlargement of various lymph nodes, and thymus, both associated with neoplasms of the hemolymphoreticular system, enlargement of the spleen, associated with neoplasia and amyloidosis and increased extramedullary haematopoiesis. The low incidence of observed liver enlargements was associated with neoplasia and amyloidosis. However, none of these findings were dose-dependent.

In mice sacrificed at termination the following changes were observed: Kidney surface rough/uneven in high dose males, discoloration / enlargement of mesenteric lymph nodes in high dose females and discoloration in high dose combined sex, and enlargement of spleens in both sexes combined at the high dose were significantly higher than in control mice. Since none of these changes showed a dose-dependency, and the corresponding histopathological changes were not significantly higher in these groups, the findings were considered incidental.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

There were no treatment-related histopathological findings observed in any dose group of either sex.

In mice found dead or sacrificed moribund during the study period the following significant histopathological changes were seen (Table B.6.5-45): Cystic glands of the stomach were significantly increased in high dose males and for both sexes combined. However, the incidence of these findings was similar to historical control data and did not show a dose dependency. Therefore, these finding was considered incidental. Increased haematopoiesis was seen in the bone (femur) of high dose males, mid- and high-dose animals combined sex.

Cell debris in tubules of epididymides was increased in mid dose males and the incidence of sub-capsular cell hyperplasia was increased in adrenals of low dose males. In addition, the incidence of kidney nephropathy in mid-dose females, as well as the incidence of lymphocyte infiltration of epididymides in mid dose males was significantly decreased. All these findings were also observed at lower doses and/or were not dose dependent. Thus, these findings were also considered incidental. Furthermore, it is necessary to consider the frequency of this finding in animals surviving to scheduled termination.

In mice sacrificed at termination the following more frequent observed changes were observed (Table B.6.5-45):

Cystic glands of the stomach were significantly increased in low-, mid- and high-dose males but without a dose-response.

Degenerative heart changes were higher in high-dose males and females, and significant higher when sexes were combined. Since the incidences were similar or slightly higher than in the historical controls, and since no dose-dependency was observed, this finding is considered incidental.

In mandibular lymph nodes lymphoid hyperplasia was significantly increase in low and mid-dose males and combined sex, whereas the incidence was significantly lower in high dose females.

In addition, extramedullary haematopoiesis was significantly increased in these lymph nodes at the mid-dose level in combined sex. In spleen extramedullary haematopoiesis was significantly increased in females and combined sex at the low dose level. In the absence of any dose-relation these findings, as well as several not statistically significant changes considered incidental.

Table B.6.5-45: Summary of non-neoplastic histopathological findings for dead and moribund animals

Finding	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
	0	100	1000	10000	0	100	1000	10000	0	100	1000	10000
Number examined	22	20	22	27	16	16	20	20	38	36	42	47
<i>Stomach</i>												
Cystic glands (n)	8	8	9	16	1	4	5	6	9	12	14	22+
<i>Kidney</i>												
Nephropathy (n)	9		10	12	5	1	1	3	14	8	11	15
<i>Bone (femur)</i>												
Increased haematopoiesis (n)	1	1	8+	5	0	1	2	3	1	2	10+	8+
<i>Epididymes</i>												
Cell debris in tubules (n)	0	1	4	0	--	--	--	--	--	--	--	--
<i>Epididymes</i>												
Lymphocyte infiltration (n)	4	1	0	3	--	--	--	--	--	--	--	--
<i>Heart</i>												
Degenerative changes (n)	11	14	13	16	4	2	4	1	15	16	17	17
<i>Adrenals</i>												
sub-capsular cell hyperplasia (n)	3	8+	7	10	12	11	13	15	15	19	20	25
<i>Mandibular LN</i>												
extramedullary haematopoiesis (n)	3	2	5	3	1	1	1	2	4	3	6	5

n = number of animals affected; LN = lymph node

+ significantly increased; -- not examined/determined

The number of malignant lymphoma was slightly elevated in the high dose group compared to control. This tumour of the hemolymphoreticular system is one of the most common tumours of mice accounting for the highest percentage of spontaneous tumours in this species. Therefore, the observed tumour incidence is considered incidental and not treatment-related. In addition, the incidences of this tumour varied with sex and fate (i.e. pre-terminal and terminal deaths).

Table B.6.5-46: Summary of non-neoplastic and neoplastic histopathological findings at termination

Finding	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
	0	100	1000	10000	0	100	1000	10000	0	100	1000	10000
<i>Stomach</i> (N)	28	30	28	23	34	-	-	30	62	64	58	53
Cystic glands (n)	9	19+	22+	17+	22	-	-	19	31	-	-	36
<i>Kidney</i> (N)	28	6	4	23	34	2	1	30	62	64	58	53
Nephropathy (n)	7	4	3	6	5	2	0	2	12	6	3	8
<i>Bone (femur)</i> (N)	28	-	-	23	34	2	1	30	62	--	--	53
Increased haematopoiesis (n)	1	-	--	0	1	0	0	2				
<i>Epididymes</i> (N)	28	1	-	23	--	--	--	--	--	--	--	--
Lymphocyte infiltration (n)	0	0	--	1	--	--	--	--	--	--	--	--
<i>Mandibular LN</i> (N)	28	30	28	23	34	33	28	30	62	64	58	53
Extramedullary haematopoiesis (n)	5	7	9	9	3	9	7	4	8	16	16+	13
<i>Heart</i> (N)	28	2	--	23	34	--	-	30	62	--	--	53
Degenerative changes (n)	14	1	--	17	2	--	-	6	16	--	--	23+
<i>Adrenals</i> (N)	28	--	--	23	34	-	-	29	62	--	--	53
sub-capsular cell hyperplasia (n)	15	--	--	13	27	--	--	22	42	--	--	35
<i>Hemolymphoreticular system</i> (N)	28	30	28	23	34	34	30	30	62	64	58	53
malignant lymphoma (n)	1	3	3	6	9	10	6	13	10	13	9	19

N = number examined; n = number of animals affected; LN = lymph node

+ significantly increased; -- not examined/determined

Table B.6.5-47: Incidences of malignant lymphoma and comparison with historical control

			Dietary concentration of glyphosate (ppm)							
	♂	♀	Males				Females			
			0	100	1000	10000	0	100	1000	10000
Dead & moribund										
Number examined	75	77	22	20	22	27	16	16	20	20
Number affected	20	49	9	12	13	13	9	10	13	12
Percentage affected	26.7	63.6	41.0	60.0+	59.0+	48.0	56.0	63.0	65.0	60.0
Mean %	26	61.8	--	--	--	--	--	--	--	--
Range %	0-44	0-100	--	--	--	--	--	--	--	--
			--	--	--	--	--	--	--	--
Terminal sacrifice										
Number examined	175	175	28	3028	23	34	34	30	30	28
Number affected	26	50	1	3	3	6+	9	10	6	13
Percentage affected	14.9	28.9	3.6	10.0	10.7	26.1+	26.5	29.4	20.0	43.3+
Mean %	14.8	28.8	--	--	--	--	--	--	--	--
Range %	8-24	20-43	--	--	--	--	--	--	--	--
			--	--	--	--	--	--	--	--
All fates										
Number examined	250	250	50	50	50	50	50	50	50	50
Number affected	46	99	10	15	16	19+	18	20	19	25
Percentage affected	18.4	39.6	20.0	30.0	32.0	38.0+	36.0	40.0	38.0	50.0+
Mean %	18.4	41.6	--	--	--	--	--	--	--	--
Range %	6-30	14-58	--	--	--	--	--	--	--	--

+ significantly increased; -- not examined/determined

Conclusion by the Notifiers

Based on mortality at the upper limit of the historical control range, the NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is conservatively set at 1000 ppm, corresponding to 149.7 mg/kg bw/day for males, 151.2 mg/kg bw/day for females, and 150.5 mg/kg bw/day for both sexes combined. It is concluded that Glyphosate is not carcinogenic in mice.

RMS comments

The study is considered acceptable and the NOAEL of 150 mg/kg bw/day is agreed but should be based on different considerations. In fact, mortality was slightly higher in males receiving the high dose (54% as compared to 44 % in the control) and in females which were fed the mid and high dose (40% in both vs. 32 % in the control) but these differences were not that convincing. As shown in Table B.6.5-42, mortality was at the upper edge of the historical control range. Based on additional information that can be obtained from the original study (occurrence of first pre-terminal deaths in the individual groups, slopes for cumulative mortality curves, mean survival for the animals that were found dead pre-terminally or were sacrificed moribund, investigations on causes of deaths, so far performed), it is not likely that this slightly lower survival rate in these few groups was due to glyphosate feeding.

In contrast, two pathological findings should be given more attention.

There was a more frequent occurrence of cystic glands of the stomach in male mice that becomes more clear when the incidences in animals found dead or moribund and those sacrificed at scheduled termination are combined (Table B.6.5-48). Indeed, the difference between the treated groups is not large and, taking into account the large dose spacing, a clear dose response may be doubted but, according to the study author, the incidence was

higher than the historical control data. Unfortunately, this historical control data was not given. In contrast, no increase became apparent in females.

Table B.6.5-48: Stomach cystic glands in the study by [REDACTED] (2001, ASB2012-11491) in Swiss albino mice, total incidence

Sex	Males				Females			
Dose	0	100	1000	10000	0	100	1000	10000
No. examined	50	50	50	50	50	16	20	50
Animals with cystic stomach glands	17	27	31	33	23	4	5	25

Based on this table and the statistical significance mentioned in Table B.6.5-47 for male animals, there was no NOEL in this study because it cannot be excluded that this finding was due to treatment. The clinical relevance of cystic glands of the stomach is not clear. In any case, there was no increase in severity (always minimal to mild) and, more important, the cysts formation did not progress to any other pathological lesion, even at a dose level that was 100 times higher than the lowest. Thus, this finding should not be taken into account when the NOAEL for this study is set.

As can be seen in Table B.6.5-47 and Table B.6.5-48, an increase in malignant lymphoma was noted in both the male and female groups receiving the highest dose. The incidence was statistically significantly elevated as compared to the actual control groups in this study, was above the mean values of the (relatively small) historical control and, for males, outside the historical control range. Even though malignant lymphoma is a common tumour in mice (accounting for 54.6% of all tumours in this study), it cannot be completely excluded that the higher incidence in the top dose groups were somehow related to treatment. The RMS conclusion is that there was limited evidence of a carcinogenic potential of glyphosate in this mouse strain at the very high dose level of 10000 ppm (about 1460 mg/kg bw/day for sexes combined) in this study, with male animals being more affected. The NOAEL should be set at the mid dose level of ca 150 mg/kg bw/day confirming a previous figure established by [REDACTED] (1983, TOX9552381) even though effects at higher dose levels were completely different.

2nd new long-term study in mice ([REDACTED] et al., 2009)

Reference: IIA, 5.5.3/02

Report: [REDACTED] (2009b), Glyphosate technical: Dietary Carcinogenicity Study in the Mouse

SPL Project No.: 2060-0011

Date: 2009-04-22

Guidelines: not published, ASB2012-11492
OECD 451 (1981), JMAFF guideline 2-1-15 (2005), US-EPA
OPPTS 870.4200 (1996)

Deviations: None

GLP: yes

Acceptability: See RMS comment

Dates of experimental work: 2005-10-10 - 2007-11-19

Materials and methods

Test material: Glyphosate technical

Identification: Glyphosate

Description: White crystalline solid

Lot/Batch #: H05H016A

Purity: 95.7 %

Stability of test compound: Expiry: 2008-03-25

Vehicle and/
or positive control: Diet

Test animals:

Species: Mouse

Strain: CD-1, CrI:CD-1 (ICR) BR

Source: [REDACTED]

Age: Approx. 5-6 weeks

Sex: Males and females

Weight at dosing: Males: 22 – 32 g, females: 18 – 28 g

Acclimation period: At least ten days

Diet/Food: Rat and Mouse SQC Ground diet No. 1, Special Diet
Services Limited, UK), *ad libitum*

Water: Tap water, *ad libitum*

Housing: Initially in groups of three per sex in polypropylene solid-
floor cages.

Environmental conditions: Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: at least 15/hour
12 hours light/dark cycle

In life dates: 2005-10-10 to 2007-11-19

Animal assignment and treatment:

In a carcinogenicity feeding study groups of 51 CD-1 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm (equivalent to mean achieved dose levels of 0, 84.7, 266.8 and 945.6 mg/kg bw/day) Glyphosate technical in diet. Additional 12 mice per sex, designated for veterinary controls, were housed and maintained alongside treated animals. Ten animals per sex from each group were set aside for an interim kill (toxicity assessment), which was carried out on the survivors after 39 weeks of dosing. The remaining 50 mice per sex and dose-level were dosed for a maximum of 79 weeks (carcinogenicity assessment).

Test diets were prepared prior to start of treatment and then weekly by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.

The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for achieved concentration monthly for the first six months and then every three months thereafter.

Clinical observations

A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all mice and recorded weekly. Observations for morbidity, and mortality were made twice daily. Additional unscheduled examinations were performed on animals that showed ill-health.

All surviving animals were palpated weekly for size, position and appearance of new or existing masses.

Body weight

Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. Body weights were also determined before sacrifice. Body weight data were reported only until Week 77.

Food consumption and compound intake

Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination. Food consumption data were reported only until Week 77. Food efficiency and compound intake was calculated from the recorded food consumption data.

Water consumption

Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Haematology

Blood smear samples were collected after 12 months and at termination from all animals, and from mice that were killed in extremis. Differential white cell counts were performed on all control and high-dose animals and on the animals killed in extremis.

Sacrifice and pathology

All animals that died or were killed in extremis during the conduct of the study, and all animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined from 10 mice per sex per group: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, spleen, and testes.

Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (incl. cerebrum, cerebellum and pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions incl. palpable masses, head (incl. pharynx, nasopharynx and paranasal sinuses), heart, Harderian and lacrimal glands, ileum, jejunum, kidneys, larynx, liver and gall bladder, lungs (with bronchi), mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, preputial gland,

prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of the liver, lungs and kidneys, as well as gross macroscopic lesions and palpable masses from low and intermediate dose groups at termination were examined microscopically.

Statistics

All data were summarised in tabular form and analysed by computerised analysis using Provantis™ Tables and Statistics Module. For each variable the of variance incorporating Student's t-test and F-test. For each variable the most suitable transformation of data was found, the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANOVA and Bartlett's test. The lowest treatment-related significant effects were determined using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no response is found, but the data showed non-homogeneity of means, data were further analysed by a stepwise Dunnet (parametric) or Steel (non-parametric) test to determine significant differences from control. If required, pair-wise tests are performed using Students t-test (parametric) or the Mann-Whitney U test (non-parametric).

The levels of probability chosen as significant were $p < 0.01^{**}$ and $p < 0.05^{*}$.

Histopathology data were analysed using Chi squared analysis (differences in the incidence of lesions occurring with an overall frequency of 1 or greater) and the Kruskal-Wallis one-way non-parametric analysis of variance (comparison of severity grades).

The levels of probability chosen as significant were $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.1$.

Results and discussion

Analysis of dose formulations

Analyses for homogeneity and stability indicated that the dose preparations were homogeneous and stable for at least six weeks. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within $\pm 5\%$ of the nominal concentration for all but 1 sample (500 ppm –level), which was $+10\%$ of the nominal concentration.

The group mean achieved doses are summarised below.

Table B.6.5-49: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Achieved dose level (mg/kg bw/day)*				
		Males		Females		Overall mean
		Mean	Range	Mean	Range	
1 (control)	0					
2 (low)	500	71.4	33 – 104	97.9	55 – 155	84.7
3 (mid)	1500	234.2	101 – 365	299.5	176 – 466	266.8
4 (high)	5000	810	461 - 1143	1081.2	610 - 1728	945.6

* based on actual food intake and body weight data

The results show a higher test material intake for females when compared to males for each dose level. Highest intakes were achieved within the first few treatment weeks, with subsequent decline thereafter. The mean intake for each dose group (sexes combined) is therefore 84.7, 266.8 and 945.6 mg/kg bw/day for 500, 1500, and 5000 ppm, respectively.

Mortality

No treatment-related effects on the deaths occurred during the study, as well as no treatment-related effects on the time of death. From three male mice that were killed in extremis, examination results suggest that the morbidity of these animals was due to fighting between cage mates.

Table B.6.5-50: Cumulated mortalities after 78-week dietary exposure to Glyphosate technical

	Dose group (ppm)			
Sex	0	500	1500	5000
Male	12 (6)	10 (8)	12 (6)	16 (6)
Female	14 (10)	13 (7)	13 (10)	11 (8)

(): number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table B.6.5-51: Percentage survival at termination after 78-week dietary exposure to glyphosate technical

	Dose group (ppm)			
Sex	0	500	1500	5000
Male	76	80	76	69
Female	73	75	75	78

Clinical observations

There were no significant treatment-related clinical signs of toxicity observed.

There were no trends in the proportion of palpable masses observed during the study period. A significant proportion observed showed evidence for regression before the animal reached the point of death or termination. Based on the results (see Table B.6.5-52) no treatment-related effect on the development of palpable masses is seen for either sex. The slight increase in the mean number of masses per animal for high-dose females and mid-dose males was considered a coincidence. The median time to appearance of palpable masses was comparable for all dose groups of either sex.

Table B.6.5-52: Group summary of palpable masses

Dose	Total number of animals in group		Number of animals with palpable masses		Total number of masses per group		Mean number of masses per animal		Median time (weeks) to appearance of masses	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0	51	51	28	23	45	38	0.88	0.75	42.00	45.75
500	51	51	32	28	49	49	0.96	0.96	42.00	46.08
1500	51	51	39	23	60	38	1.20	0.75	42.43	44.83
5000	51	51	25	23	49	51	0.96	1.00	41.67	42.50

Body weight

There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

Food consumption and compound intake

There were no treatment-related effects on food consumption for either sex noted during the study.

Water consumption

There were no treatment-related effects on water consumption for either sex noted during the study.

Haematology

There were no significance differences in the proportions of white blood cell counts for either sex at both 12 and 18 month.

Necropsy

Gross pathology

There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

There were no treatment-related histopathological findings observed in any dose group of either sex.

Conclusion by the Notifiers

Based on the study results the NOEL and NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is 810 mg/kg bw/day for males, and 1081 mg/kg bw/day for females. It is concluded that Glyphosate technical is not carcinogenic in mice.

RMS comments

The study is considered acceptable and setting of the NOAEL at the highest dose level of 5000 ppm (equivalent to 810 mg/kg bw/day in males and 1081 mg/kg bw/day in females) is supported. Indeed, there was not evidence for carcinogenicity up to this dose level and the very comprehensive ranges of tissues that were examined histologically does not suggest an increase in any non-neoplastic pathological lesion. Survival and growth of the animals were not affected. However, the dose levels chosen, although sufficiently high for a study of this type, were much lower than in other long-term studies with glyphosate in mice.

It was noted that histological examination of salivary glands covered submaxillary, sublingual and parotid glands. However, no lesions similar to those found by Chan and Mahler (1992, TOX 9551954, see B.6.3.2) in another mouse strain following administration of glyphosate over 90 days at higher doses were reported.

There was no increase in malignant lymphoma.

3d new long-term study in mice (██████████ 1997)

Reference: IIA, 5.5.3/03

Report: ██████████ (1997)

HR-001: 18-Month Oral Oncogenicity Study in Mice.

██

Data owner: Arysta LifeScience

Study No.: [REDACTED] 94-0151
Date: 1997-06-18
Not published, ASB2012-11493
Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985
 U.S. EPA FIFRA Guidelines Subdivision F, 1984
 OECD 451 (1981).
Deviations: None
GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 1995-02-21 to 1996-09-06

Materials and methods

Test material: Glyphosate technical
Identification: HR-001
Description: Solid crystals
Lot/Batch #: T-941209 T-950308
Purity: 97.56 % 94.61 %
Stability of test compound: Not mentioned in the report
Vehicle and/or positive control: Diet
Test animals:
Species: Mouse
Strain: SPF ICR (Crj:CD-1)
Source: [REDACTED]
Age: 5 weeks
Sex: Males and females
Weight at dosing: Males: 15 – 25 g, females: 14 – 23 g
Acclimation period: 9 days in males; 7 days in females
Diet/Food: Certified diet MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*
Water: Filtered and sterilized water, *ad libitum*
Housing: In groups of four per sex in aluminium cages with wiremesh floors
Environmental conditions: Temperature: 24 ± 2°C
 Humidity: 55 ± 15%
 Air changes: 15/hour
 12 hours light/dark cycle

In life dates: 1995-02-21 to 1996-09-06

Animal assignment and treatment:

Groups of 50 males and 50 females Specific –Pathogen-Free (SPF) ICR (Crj : CD-1) mice received the test material by incorporating it into the basal diet at a level of 0, 1 600, 8 000 or 40 000 ppm for a period of 18 months.

Clinical observations

All animals were conducted a cage-side observation daily for clinical signs and their deaths during the study. In addition, a detailed examination including palpation of the body was performed at least once a week. Moribund animals showing marked debility were euthanized by exsanguinations under deep ether anesthesia and necropsied when an unfavourable prognosis was predicted. Dead animals were taken from the cage as soon as possible after discovery to minimize the loss of tissues by cannibalism and necropsied.

Mortality was expressed as ratios of cumulative number of animals found dead or killed in extremis to effective number of animal group.

Body weight

Individual body weights were recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 76. Body weights were also measured at week 78, at the end of treatment, and used for calculation of relative organ weights. Group mean body weights were calculated at each measurement.

Food consumption and compound intake

Food consumption by each cage was recorded for a period of 3 or 4 consecutive days weekly during the first 13 weeks and every 4 weeks from week 16 to 76. Food efficiency and compound intake was calculated from the recorded food consumption data.

Haematology

Blood smear samples were collected at week 52 and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.

Sacrifice and pathology

All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately.

All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.

The following organ weights were determined from 10 mice per sex per group: brain, adrenals, kidneys, spleen, liver and gall bladder, ovaries, and testes.

Tissue samples were taken from each mice from the following organs and preserved in 10% buffered neutral formalin: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, esophagus, stomach, liver with gallbladder, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, pharynx, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of gross lesions and masses from all mice were examined microscopically. The following tissues were examined: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, esophagus, stomach, liver with gallbladder, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions

Statistics

Body weight, food consumption and organ weights were evaluated by Bartlett's test for homogeneity of intra group variances. When group variances were homogenous, a parametric analysis of variance of a one way layout type was conducted to determine if any statistical differences exist among groups. When the analysis of variance was significant, Dunnett's or Scheffe's multiple comparison test was applied. When the group variance were heterogeneous, the data were evaluated by Kruskal-Wallis non-parametric analysis of variance. When significant Dunnett type mean rank test or Scheffe's type mean rank test was applied.

Mortality was assessed by a life table analysis.

Urinalysis were analyzed by Mann-Whitney's U test to compare data between the treatment groups and the controls.

Mann-Whitney's U test was used to analyze difference of the differential leukocyte counts between the high dose groups and the controls. For comparison of the data from all groups, Dunnett's and Scheffe's multiple comparison test was applied. The data from males killed in extremis during the treatment were examined by Mann-Whitney's U test.

Fisher's exact probability test was used to analyze the data of clinical signs and incidences of gross lesions at necropsy and histopathological lesions.

Results and discussion

Analysis of dose formulations

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss 8.37 %. Homogeneity of the test substance in diet was analysed on the samples taken from the top, middle, and bottom portion of the mixer. The coefficient of variation for each test diet was within 5.2 % or less. The results indicated that homogeneity of the test substance in diet was satisfactory in each test diet.

In order to verify concentration of the test substance in test diets, every batch of test diet was analysed during the treatment period. Mean concentration of the test substance in test diet at a nominal level of 1 600, 8 000 or 40 000 ppm was $1\,561 \pm 86.7$, $7\,790 \pm 394.4$ or $38\,783 \pm 1\,655.0$ (mean + standard deviation) ppm, respectively. The values were within 97-98 % of the target concentrations and satisfied the acceptable limit of concentration for test substance.

Mortality

No significant differences were noted for mortality between the treated groups and the respective control of either sex. Cumulative mortality of each group of either sex is shown in the following table:

Table B.6.5-53: Final mortality at termination of treatment (%)

Dose group (ppm)	Male	Female
0	24/50 (48)	18/50 (36)
1 600	16/50 (32)	14/50 (28)
8 000	23/50 (46)	10/50 (20)
40 000	21/50 (42)	15/50 (30)

Clinical observations

Statistically significant changes in clinical signs observed in the treated groups of either sex are shown in the following table:

Table B.6.5-54: Statistically significant changes in clinical signs:

	Male				Female			
	Dose group (ppm)							
	0	1 600	8 000	40 000	0	1 600	8 000	40 000
Number of animals examined	50	50	50	50	50	50	50	50
Perinasal region : tactile hair loss	0	3	3	6*	5	13*	9	8
Anus : mass(es)	0	0	0	8**	0	0	0	0
Integument :								
Wound	22	16	20	6*	3	0	0	0
Erosion/Ulcer	9	5	12	8	16	4**	4**	2**
Swelling	16	6*	13	9	6	2	0	1
Mass(es)	15	13	13	10	13	11	9	4*
Pale-colored skin	2	3	6	10*	4	2	6	6
Hair loss	11	12	21*	12	22	23	18	14
Wetted fur	11	9	7	4*	1	1	1	1

* : $p < 0.05$; ** , $p < 0.01$ (Fisher's exact probability test).

In the 40 000 ppm group, males showed increased incidences of tactile hair loss, pale-colored skin, and mass(es) of anus and decreases of wound and wetted fur. In females of this group decreased incidences were observed in ulcer/erosion and mass(es) of skin. Although, in addition to these signs, loose stool was observed in the cages of both sexes beginning at week 21 in males and 20 in females, the group housing failed to identify which animal excreted the loose stool.

In the 8 000 ppm group, males showed an increased incidence in hair loss of the skin and females represented decreases in ulcer/erosion and swelling of the skin.

In the 1 600 ppm group, males showed a decrease in swelling of the skin and females represented an increase in tactile hair loss as well as a decrease in ulcer/erosion of the skin.

None of the observed effects seems to be dose-related. Whatever the dose tested, females were more sensitive to erosion/ulcer of the integument than males.

Body weight

In the 40 000 ppm group, males and females showed retarded growth during the treatment manifesting significantly lowered weights at weeks 16 to 36 in males and at weeks 6 and thereafter in females compared to the respective control. At the end of treatment, mean average weights were 93 % and 86 % of the respective control in males and females, respectively.

In the 8 000 ppm group, females showed significantly decreased weights at week 6 and weeks 9 to 24 compared to the control and the final mean average weight was 92% of the control at the end of the treatment, while growth rate in males was comparable to the control.

In the 1 600 ppm group, males and females showed similar growth curves to the controls during the treatment period.

Effects on the body weight were more important in females than in males. These effects were durable in the 40 000 ppm female group whereas they were stopped at week 36 in the male group of the same treatment dose. Sporadic effects were observed in the 8000 ppm female group. No significant effects were seen in the 1600 ppm male and female groups.

Food consumption and compound intake

In the 40 000 ppm group, males showed significant depressions in food consumption at weeks 1 and 68, revealing an overall group mean food consumption at 94% of the control during the treatment period. Females in this group also showed significantly decreased food

consumption at weeks 1, 4, 8, 12, 20, 28, 40, 48 and 68, revealing an overall group mean food consumption at 93% of the control during the treatment period.

In the 8 000 ppm group, females showed significantly lowered food consumption at weeks 28, 40, and 68 compared to the control manifesting an overall group mean food consumption at 96 % of the control. Whereas, food consumption in males was comparable to the control during the treatment period.

No statistically significant effects was observed in the 1600 ppm group either in males or females.

The food consumption depressions were more important in female than in males. They were not time-related

Overall average chemical intake in each treated group of either sex was calculated from food consumption and nominal concentration as shown in the following table:

Table B.6.5-55: Calculated test substance intake in mg/kg bw/day:

Dose level (ppm)	Dose level (mg/kg bw/day)	
	Male	Female
1 600	165.0	153.2
8 000	838.1	786.8
40 000	4348	4116

Haematology

Statistically significant changes in differential leucocyte counts observed in the treated group of either sex are shown in the following table.

Table B.6.5-56: Statistically significant changes in haematology parameters:

Parameter	Sex	Fate of animals ^a	Dose group (ppm)		
			1600	8000	40000
Lymphocytes	Males	Ke	ND ^b		↑ 172
	Females	tk			↑ ^c 163
Neutrophil (segmented)	Males	ke	ND		↓ 81

Numbers in the above table show values in the treated groups when the corresponding value in the control group is 100.

a: ke, killed in extremis; tk, terminal kill

b: ND, not determined

c: Dunnett's or Scheffe's multiple comparison test

↓↑: Mann-Whitney's U test

In the 40000 ppm group, males killed *in extremis* during the treatment period showed an increase of lymphocytes in differential leukocyte counts and a decrease of neutrophil (segmented form). In females of this group, differential count of lymphocytes was significantly increased at week 78.

There were no significant differences in differential leukocyte counts at other intervals of examination in the 40000 ppm group of both sexes, males killed *in extremis* in the 8000 ppm group and females at week 78 in the 8000 and 1600 ppm groups compared to the controls. No significant treatment-related effects were conceived in morphology of the leukocytes.

Necropsy

Gross pathology

Statistically significant changes in incidence of macroscopic lesions observed in the treated groups of either sex are shown in the following table.

Table B.6.5-57: Statistically significant changes in macroscopic lesions:

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
External appearance: Loss of tactile hair	0	0	1	5*	4	8	8	0*
Soiled fur on external genital region	9	7	2*	6	0	0	0	0
Spleen: Swelling	5	1*	4	2	7	2	3	3
Lung: Mass(es)	4	12	11*	9	8	6	18	8
Cecum: Distention	0	0	0	11**	0	0	0	16**
Kidney: Cyst(s)	4	4	2	0*	2	0	4	1
Uterus: Cyst(s)	-	-	-	-	6	2	2	0*
Skin: Loss of hair	1	4	7*	6	8	11	16	5
Ke/fd (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)
Lymph nodes (mesenteric): Swelling	0	2	0	5*	1	2	1	4
Lymph nodes (others): Swelling	5	2	4	9	0	3	4*	4*
Kidney: Coarse surface	4	2	1	1	6	3	0*	4
Skin: Loss of hair	5	4	7	4	11	5	2*	4
Wound	6	2	3	0*	0	0	0	0
Ulcer/Erosion	6	3	4	6	5	3	0	0
All (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
External appearance: Loss of tactile hair	0	0	1	6*	5	11	9	3
Lymph nodes (cervical): Swelling	5	3	6	9	12	6	4*	7
Lymph nodes (mesenteric): Swelling	0	2	0	6*	3	2	1	5
Spleen: Swelling	16	4**	12	14	17	8*	8*	10
Lung: Mass(es)	9	14	17	15	10	8	20*	11
Cecum: Distention	0	0	0	14**	0	0	0	18**
Anus: Anal prolapse	0	0	0	5*	0	0	0	0
Kidney: Pale in color	6	2	4	2	7	4	1*	4
Coarse surface	6	2	2	1	7	4	0**	5
Testis: Atrophy	5	2	5	0*	-	-	-	-
Uterus: Cyst(s)	-	-	-	-	6	2	2	0*
Eye: Opacity	1	1	5	2	5	1	0*	0*
Auricle: Partial amputation	6	2	1	0*	4	2	0	1
Skin: Loss of hair	6	8	14*	10	19	16	18	9*
Wound	9	3	3	1*	0	0	0	0
Ulcer/Erosion	7	4	9	6	8	3	1*	0**
Swelling	7	1*	3	1*	3	0	0	0

Tk: Terminal kill

Ke/fd: Killed in extremis or found dead

All: All animals examined

(N=): Number of animals examined

*, p<0.05 (Fisher's exact probability test); **, p<0.01

In the 40000 ppm group, males and females showed significant increases in incidence of distention of the cecum at terminal kill after 78 weeks of treatment. Significant increases in incidence of the lesion were also noted in all animals examined recording 28 % (14/50) in

males and 36 % (18/50) in females. Distended cecum was filled with loose stool-like materials. In addition, males showed an increase in loss of tactile hair and a decrease of cyst(s) in the kidney in those necropsied at terminal kill, and an increase of swelling in the lymph nodes (mesenteric) and a decrease of wound in the skin in those killed in extremis or found dead during the treatment period when compared to the controls. Among these, significant differences in incidence were also noted in all animals examined for increases in loss of tactile hair and swelling of the lymph nodes (mesenteric) and a decrease in wound in the skin. Moreover, significant differences in incidence were also noted in all animals examined for an increase in anal prolapse of the anus and decreases in atrophy of the testis, partial amputation of the auricle, and swelling of the skin. Females showed decreases in loss of tactile hair and cyst(s) of the uterus in those necropsied at terminal kill and an increase in swelling of the lymph nodes (others) and a decrease in ulcer/erosion of the skin in those killed in extremis or found dead during the treatment period. Among these, significant differences in incidence were noted in all animals examined for decreases in cyst(s) of the uterus and ulcer/erosion of the skin. Moreover, significant differences in incidence were also noted in all animals examined for decreases in opacity of the eye and loss of hair of the skin.

In the 8000 ppm group, males showed increases in mass(es) of the lung and loss of hair of skin and a decrease in soiled fur on external genital region in those necropsied at terminal kill when compared to the control. An increased incidence was also noted in all animals examined for loss of hair of the skin. Females killed in extremis or found dead during the treatment period in this group showed an increase in swelling of the lymph nodes (others) and decreases in coarse surface of the kidney and loss of hair of the skin. Moreover, significant differences in incidence were noted in all animals for an increase in mass(es) of the lung and decreases in swelling of the lymph nodes (cervical) and spleen, pale in color and coarse surface of the kidney, opacity of the eye, and ulcer/erosion of the skin.

In the 1600 ppm group, males showed decreased incidences in swelling of the spleen in those necropsied at terminal kill and in all animals examined and in swelling of the skin in all animals examined, while females disclosed a decreased incidence in swelling of the spleen in all animals examined.

Organ weights

In the 40000 ppm group, males and females showed significant increases in absolute and relative weights of the cecum. The percentages of the values to those of the respective control were 173 % in males and 187% in females for absolute weight, respectively, and 174 % and 212 % for relative weight, respectively. In females, relative weight of the kidney was also increased significantly at a level of 111 % of the control.

Histopathology

Neoplastic lesions

The table below shows neoplastic lesions in the treated groups of either sex with statistically significant differences in incidence from those of the controls.

Table B.6.5-58: Statistically significant changes in histopathology findings:

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
<i>78tk</i> (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
Hematopoietic & Lymphatic system: General: Malignant lymphoma	0	0	1	5*	4	8	8	0*

Tk: Terminal kill

(N=): Number of animals examined

*, p<0.05 (Fisher's exact probability test)

As to neoplastic lesions, the incidence of malignant lymphoma was significantly decreased in females of the 1600 ppm group necropsied at terminal kill compared to the control. Neither increases in incidence nor nearly occurrences compared to the controls were noted for neoplastic lesions in the treated groups of both sexes.

Non-neoplastic lesions

Statistically significant changes in incidence of non-neoplastic lesions observed in the treated groups of either sex are shown in the following table.

Statistically significant changes in incidence of non-neoplastic lesions:

Table B.6.5-59: Statistically significant changes in non-neoplastic lesions:

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
<i>78tk</i> (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
Spleen: Increased extramedullary hematopoiesis	5	2	4	3	6	5	1*	4
Liver: Micro-granuloma	1	5	5	4	15	16	14	7*
Kidney: Cortical cyst(s)	9	6	9	0*	2	1	5	0
Tibio-femoral joint: Proliferation of cartilaginous tissue	14	17	11	15	18	14	11*	15
<i>Ke/fd</i> (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)
Bone marrow (femur): Increased hematopoiesis	6	3	7	6	7	1*	1	2
Lymph nodes (cervical): Plasma cell hyperplasia	6	1	5	4	5	3	0	0*
Spleen: Amyloid deposition	2	3	2	0	8	3	0*	1*
Small intestine: Amyloid deposition	1	1	1	0	5	0*	0	2
Liver: Amyloid deposition	3	3	2	0	10	3	0**	1**
Thyroid: Amyloid deposition	2	2	2	0	8	1*	0*	2
Parathyroid: Amyloid deposition	1	1	2	0	7	1	0*	2
Skin: Wound	9	5	9	4	9	5	1*	3
<i>All</i> (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Bone marrow (femur): Increased hematopoiesis	9	3	10	10	9	2*	2*	2*
Bone marrow (sternum): Increased hematopoiesis	9	3	9	10	9	3	2*	2*
Bone marrow (Vertebra): Increased hematopoiesis	9	3	10	10	9	3	2*	2*
Lymph nodes (cervical): Plasma cell hyperplasia	6	2	8	5	8	3	1*	0*
Lymph nodes (mesenteric): Myeloid cell aggregation	5	0*	3	2	4	1	1	1
Spleen: Increased extramedullary hematopoiesis	20	7*	14	14	13	10	5*	9

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
Amyloid deposition	3	3	4	0	10	3*	0**	1**
Lung: Alveolar epithelial cell hyperplasia	0	5*	1	1	3	4	5	5
Small intestine: Amyloid deposition	2	1	1	0	8	0**	0**	
Liver: Micro-granuloma	1	6	5	5	16	16	14	7*
Amyloid deposition	5	3	4	0*	12	3*	0**	1**
Kidney: Cortical cyst(s)	10	8	13	2*	5	1		0*
Glomerular amyloidosis	1	1	2	0	7	2	0**	2
Uterus: Amyloid deposition	-	-	-	-	6	0*	0*	1
Thyroid: Amyloid deposition	3	2	4	0	11	1**	0**	2**
Parathyroid ^c : Amyloid deposition	2	1	4	0	10	**	0**	2*
Eye: Cataract	4	5	5	5	5	2	0*	2
Skin: Skin subcutaneous abscess	3	1	2	5	5	1	0*	1

Tk: Terminal kill

Ke/fd: Killed in extremis or found dead

All: All animals examined

(N=): Number of animals examined

*, $p < 0.05$; **, $p < 0.01$ (Fisher's exact probability test)

c: The number animals examined in the control, 1600, 8000 or 40000 ppm groups were 46, 48, 48 or 46 in males and 48, 48, 50 or 49 in females, respectively.

In the 40000 ppm group, males showed significant decreases in incidence of amyloid deposition in the liver in all animals examined and cyst(s) in the kidney in those necropsied at terminal kill and in all animals examined, when compared to the control. In these males, erosion/ulcer in the anus was observed in a total of 8 animals including 6 cases killed in extremis or found dead during the treatment period and 2 cases necropsied at terminal kill. There was even a large abscess in one case. Among these, regressive hyperplasia of mucous epithelium of the large intestine was seen in 2 cases with severe lesions in the anus. However, as the histopathological examinations were carried out only on the anus which were observed macroscopic lesions, the incidence of erosion/ulcer in the anus was not assessed by a statistical method. In females of this group, statistical significant decreases in incidence were noted in all animals examined as follows; increase hematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), cyst(s) in the kidney, micro-granuloma in the liver, and amyloid deposition in the spleen, liver, thyroid, and parathyroid. Among these, significant decreases in incidence were also noted for micro-granuloma in the liver in those necropsied at terminal kill and plasma cell hyperplasia in the lymph nodes (cervical) and amyloid deposition in the spleen and liver in those killed in extremis or found dead during the treatment period.

In the 8000 ppm group, although males did not show any non-neoplastic lesions with statistically significant differences in incidence from the control, females disclosed significant decreases in incidence of proliferation of cartilaginous tissue in the tibio-femoral joint in those necropsied at terminal kill, wound in the skin in those killed in extremis or found dead during the treatment period, and subcutaneous abscess in the skin in all animals examined. In addition, significant decreases in incidence, when compared to the control, were observed in all animals examined as follows; increase hematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), extramedullary hematopoiesis in the spleen, amyloid deposition in the spleen, small intestine, liver, kidney (glomerular amyloidosis), uterus, thyroid, and parathyroid, and cataract in the eye. Among these, the incidences of extramedullary hematopoiesis in the spleen in those necropsied at

terminal kill and amyloid deposition in the spleen, liver, thyroid, and parathyroid in those killed in extremis or found dead during the treatment period were also decreased significantly.

In the 1600 ppm group, males in all animals examined showed a significant increase in incidence of alveolar epithelial cell hyperplasia in the lung and decreases in incidence of myeloid cell aggregation in the lymph nodes (mesentery) and extramedullary hematopoiesis in the spleen. In females of this group, the incidences in all animals examined were decreased significantly in increased hematopoiesis in bone marrow (femur) and amyloid deposition in the spleen, small intestine, liver, uterus, thyroid, and parathyroid. Among these, significantly decreased incidences were also noted for increased hematopoiesis in bone marrow (femur) and amyloid deposition in the small intestine and thyroid in those killed in extremis or found dead during the treatment period.

Conclusion by the Notifiers

Based on the results, no oncogenic potential was observed in glyphosate after treated to mice at a dietary level of as high as 40 000 ppm for a period of 18 months (78 weeks).

RMS comments

The study is considered acceptable. Based on the effects in female mice on food consumption and body weight gain at the mid dose level of 8000 ppm, the lowest dose of 1600 ppm (ca. 153 mg/kg bw/day) is considered the NOAEL in this study. In contrast, the masses in lung mentioned in the dossier were not dose-related and there was no convincing evidence of lymph node swelling.

Male mice appeared less vulnerable.

There was an apparent error with regard to carcinogenicity:

If compared to the original study report (Text table 6, p. 48), the figures given by GTF in Table B.6.5-60 (6.5-58 in the dossier) are wrong. The precise figures are as follows:

Table B.6.5-60: Incidence of malignant lymphoma at terminal sacrifice in the study by Sugimoto (1997, ASB2012-11493), revised

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78 Tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
Hematopoietic & Lymphatic system:	0	0	1	5*	4	8	8	0*
General: Malignant lymphoma	0	0	0	2	4	0*	5	3

Tk: Terminal kill

(N=): Number of animals examined

* $p < 0.05$ (Fisher's exact probability test)

If these figures are used, the paragraph that is written below in the original text becomes clear.

Total incidence of malignant lymphoma (including animals that were prematurely found dead or had to be killed in extremis) is given in the following Table B.6.5-61 that was introduced by the RMS.

Table B.6.5-61: Total incidence of malignant lymphoma in the study by Sugimoto (1997)

Sex	Male				Female			
<i>Dose group (ppm)</i>	0	1600	8000	40000	0	1600	8000	40000
<i>No. examined</i>	50	50	50	50	50	50	50	50
<i>Hematopoietic & Lymphatic system:</i>								
<i>General: Malignant lymphoma</i>	2	2	0	6	6	4	8	

The slight increase in high dose males was not statistically significant. Unfortunately, no historical control data for malignant lymphoma from the performing laboratory was provided.

Furthermore, it was noted that the study director was actually Mika Kinoshita. The report writer (Kayoko Sugimoto) was as a pathologist involved in histopathological examination.

B.6.5.3 Published data on carcinogenicity (released since 2000)

Epidemiology studies

A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the specificity of their conclusions regarding pesticides in general, 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. Publications suggesting glyphosate is associated with any cancer outcome are discussed below.

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 speculation if exposures are not identifiable. Pivotal to the understanding of glyphosate exposure are data published by Acquavella et al. (2004, ASB2012-11528; 2005, ASB2012-11530), which quantified human systemic glyphosate exposure levels in farmer applicators and their families. The geometric mean systemic dose for farmers applying glyphosate, some of whom applied glyphosate to areas up to 400 acres, was 0.0001 mg/kg/day, approximately 0.03 % of the EU glyphosate acceptable operator exposure Level (AOEL) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). The highest systemic dose, skewed well above the geometric mean, was 0.004 mg/kg/day, which is 1.95 % EU glyphosate AOEL according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191) and 1.3 % of the current EU glyphosate acceptable daily intake (ADI) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). Even lower systemic doses were determined for spouses and children, 0.00004 mg/kg and 0.0008 mg/kg, respectively. Multiple carcinogenicity studies have since been conducted by numerous glyphosate registrants demonstrating NOAELs of at least ten-fold higher than the highest dose tested in the study driving the current EU ADI calculation.

The largest epidemiological study of pesticide exposure and health outcomes in the United States is the Agricultural Health Study (AHS), which included glyphosate. Dozens of publications have resulted from data generated in this study of approximately 57,000 enrolled farmer 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 leukemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos et al. (2005, ASB2012-11605) reported AHS data evaluating glyphosate use and multiple cancer endpoints; no association was noted for glyphosate with all cancers, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, non-Hodgkin's lymphoma (NHL) and leukemia. In an earlier publication based on another data set, however, De Roos et al., (2003, ASB2012-11606) reported an association between NHL and glyphosate use. McDuffie et al. (2001, ASB2011-364) reported a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. Blair et al. (2009, ASB2012-11566) 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. The AHS publication reporting this refers to a "suggested association" between glyphosate use and multiple myeloma (De Roos et al., 2005, ASB2012-11605), yet it did not demonstrate significant increase in relative risk for multiple myeloma. Both De Roos papers will be discussed in more detail below. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009, ASB2012-11623) specifically references De Roos (2005 ASB2012-11605) as providing no observed incidents of cancers of any type being 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 that higher positive associations observed for proxy respondents with glyphosate and several other pesticides, and suggested perhaps more accurate reporting of proxies for cases, and underreporting by proxies for controls; proxy respondents were spouses in 62 % of cases versus 45 % of controls, lending to lower reported incidents in the control group.

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, chlorothalanil and "others". No association was noted for paternal exposures, but elevated leukaemias were associated with maternal exposures to "other pesticides" during pregnancy. Similarly, glyphosate is captured under "other pesticides" being associated with NHL by Fritschi et al. (2005, ASB2012-11624) and therefore should not be interpreted as an association with glyphosate.

Some further epidemiologic studies are 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, Hardell 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 for a heterogeneous category of other herbicides than above, not for glyphosate. No information is given about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc.). In all, the above mentioned limitations of the publication from Hardell and Eriksson (1999, ASB2012-11838) are also the limitations of the publication from 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 of NHL. However, no association between NHL and glyphosate can be made on basis of this study. No information was given about exposure duration, used glyphosate products, exposure duration 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.

The following epidemiology publications report a lack of 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 of 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 esophageal 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.

In summarizing AHS publications, 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, leukemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, esophagus cancer and thyroid cancer.

Animal studies

Just recently (*i.e.*, after submission of the GTF dossier), a two-year study in rats was published (Séralini *et al.*, 2012, ASB2012-15514). Its main objective was to show a possible impact of long-term feeding of genetically modified (and glyphosate-treated) 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 remarkable 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 one of a subchronic study. Such a small number of animals is not appropriate for a long-term study because age-related changes cannot be adequately taken into account. Following the receipt of contributions from many MS authorities, a comprehensive critical assessment was published by EFSA (2012, ASB2012-15513, EFSA Journal, 2012, 10 (11), 2986). The conclusion was that "the currently available evidence does not impact on the ongoing re-evaluation of glyphosate...". This opinion on the Séralini study is agreed with and supported by the RMS.

In reaction to this publication a large number of letters was sent to the editor: Barale-Thomas (2012, ASB2013-10998), Berry (2012, ASB2013-10988), Grunewald (2012, ASB2013-11001), Hammond et al. (2012, ASB2013-10995), Heinemann (2012, ASB2013-10987), Langridge (2012, ASB2013-10986), Ollivier (2012, ASB2013-11000), Panchin (2013, ASB2013-10937), Piliu (2012, ASB2013-10992), Schorsch (2013, ASB2013-10996), Tester (2012, ASB2013-10994), Tien & Huy (2012, ASB2013-10984), Trewavas (2012, ASB2013-10989), Tribe (2012, ASB2013-10997), Wager (2012, ASB2013-10993), de Souza (2012, ASB2013-10999).

Chruszielska et al. (2000, ASB2013-9829) published a combined long term toxicity and carcinogenicity study in rats. The active substance glyphosate was used in the study and the study was performed on basis of OECD guideline 453. The number of animals per dose group and sex (85 animals) was even higher than required in guideline 453. Therefore, the study is considered to be relevant. No carcinogenic effects have been registered in the study.

George *et al.*, (2010, ASB2012-11829) used a 2-stage cancer model in mice to evaluate a glyphosate formulation for tumor promotion. A known tumor promoter, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) was used as a positive control and for comparison with glyphosate effects after exposure to a tumor initiator, 7, 12-dimethylbenz[a]anthracene. Proteomics were

later applied to extrapolate a basis for glyphosate formulation tumor promotion. The results are considered by the authors to indicate a tumor promoting potential of glyphosate. However, the formulation Roundup was used in the study and not the active substance glyphosate. Furthermore, the up- and down-regulation of protein expression is not sufficient to prove a carcinogenic effect.

Author(s)	Year	Study title
Hardell, L. Eriksson, M.	1999	A Case-Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides. Cancer, Volume: 85, Number: 6, Pages: 1353-1360 ASB2012-11838

Abstract*

Background. The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodeficient conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL.

Methods. A population-based case-control study in northern and middle Sweden encompassing 442 cases and twice as many controls was performed. Exposure data were ascertained by comprehensive questionnaires, and the questionnaires were supplemented by telephone interviews. In total, 404 cases and 741 controls answered the questionnaire. Univariate and multi-variate analyses were performed with the SAS statistical data program.

Results. Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0–2.5) and fungicides (OR, 3.7; 95% CI, 1.1–13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9–2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0–6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL.

Conclusion. Exposure to herbicides in total, including phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Study prone to selection and recall bias. No evidence of relevant glyphosate exposures. Medical history was assessed, but not reported.

Relevance of study:

Not relevant (Exposure to multiple chemicals and though glyphosate exposure data were convincing (7/1145 subjects) and statistically non-significant positive associations reported.)

Klimisch code:

3

Additional comments:

Hardell and Eriksson (1999, ASB2012-11838) conducted a case control study to look for associations between reported pesticide use and non-Hodgkin's lymphoma (NHL). The study included 404 NHL cases and 741 controls. The measure of association in this study was the odds ratio (OR), a statistic that estimates of the ratio of disease rates (in this case NHL rates) for exposed and unexposed populations.

The authors reported statistically significant associations for NHL with: reported use of any herbicide (OR = 1.6), reported use of any fungicide (OR = 3.7), and reported use of 4-chloro-2-methylphenoxyacetic acid (OR = 2.7). 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 controlling for potential confounding factors, given the small number of exposed subjects.

The authors also reported a moderately elevated OR of 2.3 for glyphosate. This OR was not statistically significant and was based on only four "exposed" cases and three "exposed" controls.

This study has several important limitations: no exposure assessment, dependence on next-of-kin's recollections of study subjects' pesticide use for approximately 43 % of study subjects, potential recall bias, and the very small number of subjects who reported using specific herbicides. The latter leads to findings that are statistically imprecise. Due to the potential for bias and the statistical imprecision, the results of this study are not convincing.

Author(s)	Year	Study title
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. Leukemia & Lymphoma Volume: 43 Number: 5 Pages: 1043-1049 ASB2012-11839

Abstract*

Increased risk for non-Hodgkin's lymphoma (NHL) following exposure to certain pesticides has previously been reported. To further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL a pooled analysis was performed on two case-control studies, one on NHL and another on hairy cell leukemia (HCL), a rare subtype of NHL. The studies were population based with cases identified from cancer registry and controls from population registry. Data assessment was ascertained by questionnaires supplemented over the telephone by specially trained interviewers. The pooled analysis of NHL and HCL was based on 515 cases and 1141 controls. Increased risks in uni-variate analysis were found for subjects exposed to herbicides (OR 1.75, CI 95% 1.26-2.42), insecticides (OR 1.43, CI 95% 1.08-1.87), fungicides (OR 3.11, CI 95% 1.56-6.27) and impregnating agents (OR 1.48, CI 95% 1.11-1.96). Among herbicides, significant associations were found for glyphosate (OR 3.04, CI 95% 1.08-8.52) and 4-chloro-2-methyl phenoxyacetic acid (MCPA) (OR 2.62, CI 95% 1.40-4.88). For several categories of pesticides the highest risk was found for exposure during the latest decades before diagnosis. However, in multi-variate analyses the only significantly increased risk was for a heterogeneous category of other herbicides than above.

- Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

This publication combines the results of two previous studies by the authors on HNL (Hardell and Eriksson, 1999, ASB2012-11838) and HCL (Nordström, et al., 1998, TOX1999-687). No information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc). Study documentation is insufficient for assessment.

Relevance of study:

Not relevant (Due to reliability of data set drawn from Hardell and Eriksson, 1999, ASB2012-11838)

Klimisch code:

3

Additional comments:

This study pools data from the previously reviewed publication by Hardell and Eriksson (1999, ASB2012-11838) with data from Nordström et al. (1998, TOX1999-687). Therefore the discussion of limitations of Hardell and Eriksson (1999, ASB2012-11838) also applies to Hardell et al. (2002, ASB2012-11839) (see above).

Author(s)	Year	Study title
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 Volume: 162, Pages: 849-857 ASB2012-11624

Abstract*

Pesticide exposure may be a risk factor for non-Hodgkin's lymphoma, but it is not certain which types of pesticides are involved. A population-based case-control study was undertaken in 2000-2001 using detailed methods of assessing occupational pesticide exposure. Cases with incident non-Hodgkin's lymphoma in two Australian states (n = 694) and controls (n = 694) were chosen from Australian electoral rolls. Logistic regression was used to estimate the risks of non-Hodgkin's lymphoma associated with exposure to subgroups of pesticides after adjustment for age, sex, ethnic origin, and residence. Approximately 10 % of cases and controls had incurred pesticide exposure. Substantial exposure to any pesticide was associated with a trebling of the risk of non-Hodgkin's lymphoma (odds ratio = 3.09, 95 % confidence interval: 1.42, 6.70). Subjects with substantial exposure to organochlorines, organophosphates, and "other pesticides" (all other pesticides excluding herbicides) and herbicides other than phenoxy herbicides had similarly increased risks, although the increase was statistically significant only for "other pesticides." None of the exposure metrics (probability, level, frequency, duration, or years of exposure) were associated with non-Hodgkin's lymphoma. Analyses of the major World Health Organization subtypes of non-Hodgkin's lymphoma suggested a stronger effect for follicular lymphoma. These increases in risk of non-Hodgkin's lymphoma with substantial occupational pesticide exposure are consistent with previous work.

Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

No information about exposure duration, used glyphosate products, exposure duration and application rates. Documentation is insufficient for assessment.

Relevance of study:

Not relevant (Multiple pesticide exposures. No definitive association between NHL and glyphosate can be made.)

Klimisch code:

3

Additional comments:

No information about exposure duration, used glyphosate products, exposure duration and application rates. Only multiple pesticide exposures are reported. No association between NHL and glyphosate can be made on basis of this study.

Author(s)	Year	Study title
De Roos, A. J. Zahm, S. H. Cantor, K. P. Weisenburger, D. D. Holmes, F. F. Burnmeister, L. F. Blair, A.	2003	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occupational and Environmental Medicine Volume: 60, Number: 9, Pages: -E11 ASB2012-11606

Abstract*

Background: An increased rate of non-Hodgkin's lymphoma (NHL) has been repeatedly observed among farmers, but identification of specific exposures that explain this observation has proven difficult.

Methods: During the 1980s, the National Cancer Institute conducted three case-control studies of NHL in the midwestern United States. These pooled data were used to examine pesticide exposures in farming as risk factors for NHL in men. The large sample size (n = 3417) allowed analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides in the model, and adjusting the estimates based on a prespecified variance to make them more stable.

Results: Reported use of several individual pesticides was associated with increased NHL incidence, including organophosphate insecticides coumaphos, diazinon, and fonofos, insecticides chlordane, dieldrin, and copper acetoarsenite, and herbicides atrazine, glyphosate, and sodium chlorate. A subanalysis of these "potentially carcinogenic" pesticides suggested a positive trend of risk with exposure to increasing numbers.

Conclusion: Consideration of multiple exposures is important in accurately estimating specific effects and in evaluating realistic exposure scenarios.

Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc) were reported. Specific lymphomas are not identified (NHL captures all types of lymphoma other than Hodgkin's lymphoma). Documentation is insufficient to associate exposures with specific NHL diseases.

Relevance of study:

Not relevant (No report of identifying various types of lymphoma under the NHL umbrella; no definite association between specific NHL diseases and glyphosate can be made)

Klimisch code:

3

Additional comments:

No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc) were reported. Specific lymphomas are not identified. The reported hierarchical regression did not find a statistically significant odds ratio for ever use of glyphosate and NHL.

Author(s)	Year	Study title
De Roos, A.J. Blair, A. Rusiecki, J.A. Hoppin, J.A. Svec, M. Dosemeci, M. Sandler, D.P. Alavanja, M.C.	2005	Cancer Incidence among Glyphosate-Exposed Pesticide Applicators in the Agricultural Health Study Environmental Health Perspectives Volume: 113, Number: 1, Pages: 49-54 ASB2012-11605

Abstract*

Glyphosate is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. Although there has been little consistent evidence of genotoxicity or carcinogenicity from *in vitro* and animal studies, a few epidemiologic reports have indicated potential health effects of glyphosate. We evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrolment (1993–1997). Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which > 97% were men. In this analysis, glyphosate exposure was defined as *a*) ever personally mixed or applied products containing glyphosate; *b*) cumulative lifetime days of use, or “cumulative exposure days” (years of use × days/year); and *c*) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between glyphosate and incidence of all cancers combined and 12 relatively common cancer subtypes. Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes we studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the AHS. Given the widespread use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects, including less common cancers.

* Quoted from article

Klimisch evaluation

Reliability of study:

Reliable without restrictions

Comment:

Well documented publication. Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.

Relevance of study:

Relevant (Evaluation focussed on glyphosate, although other pesticides were also considered in the data evaluation)

Klimisch code:

2

Additional comments:

Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.

Response 1 – summary from Letter to the Editor by Farmer et al. (2005, ASB2012-11616)

Authors provided an incomplete genotoxicity review which was inconsistent with opinions of regulatory agencies and experts around the world, that glyphosate is not genotoxic. An extensive toxicology review of glyphosate was cited by the authors, mentioning a lack of carcinogenicity with glyphosate exposures, yet neglected to cite the extensive genotoxicity review in the same publication by Williams et al. (2000, ASB2012-12053)

Biological plausibility of a cancer effect should be considered in the light of exposure. Acquavella et al (2004, ASB2012-11528) reported the maximum systemic dose to resulting from application of glyphosate to areas as large as 400 acres was 0.004 mg/kg, and the geometric mean systemic dose was 0.0001 mg/kg in farmers. If these glyphosate applications and exposures continued daily over the course of a lifetime, the systemic dose would be at least 250,000-fold lower than the cancer no-effect level in rodents.

The authors were requested to further evaluate their models for confounding and selection bias in the multiple myeloma analysis.

Response 2 – summary from [REDACTED] (2007, ASB2012-11877)

Table 2 of De Roos et al. (2005, ASB2012-11605) noted 32 cases of multiple myeloma associated with “ever-use” of glyphosate and when compared with “never-use” (adjusted for age only) yielded a rate ratio of 1.1 (95 % CI 0.5-2.4). However, when the data set was adjusted for age, demographic and lifestyle factors and other pesticide use, the rate ratio increased to 2.6 (95 % CI 0.7-9.4).

The adjusted estimate merits careful inspection and can only be undertaken with access to the primary data, not made available by the authors.

Bias analysis was conducted, accounting for confounding and exposure misclassification.

Adjustment for confounders in De Roos et al. (2005, ASB2012-11605), which resulted in limiting the data set by 25 % because of missing data on the adjustment variables, likely introduced selection bias and produced the a rate ratio of 2.6 that was substantially biased.

Author(s)	Year	Study title
Eriksson, M. Hardell, L. Carlberg, M. Akerman, M.	2008	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis International Journal of Cancer Volume: 123, Pages: 1657-1663 ASB2012-11614

Abstract*

We report a population based case-control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18-74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91 %) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18-2.51. Regarding phenoxyacetic acids highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27-6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10-3.71 and with >10 years latency period OR 2.26, 95% CI 1.16-4.40. Insecticides overall gave OR 1.28, 95% CI 0.96-1.72 and impregnating agents OR 1.57, 95% CI 1.07-2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between

exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.

Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Multiple avenues for bias were introduced in study design, execution and data processing. No information about exposure duration, used glyphosate products and application rates. Other factors (i.e. smoking habits, medication etc.) were assessed but not included in the evaluation.
Relevance of study:	Relevant with reservation
Klimisch code:	3

Additional comments:

The authors (Eriksson et al. 2008, ASB2012-11614) conducted a population-based case-control study of exposure to a variety of pesticides and non-Hodgkin lymphoma (NHL), including separate analyses of histopathological categories of NHL. Study subjects were males and females, ages 18-74, living in Sweden between December 1, 1999 and April 30, 2002. The final study group included 910 cases and 1016 controls. Exposure, ascertained via an interviewer-administered questionnaire, focused on pesticide and other chemical agents, and included a total work history (although a job-exposure matrix was not used). For pesticide exposure, information on number of years, number of days per year, and approximate length of exposure per day was also obtained. A minimum of one full day of exposure was required for categorization as “exposed.”

The authors reported a statistically significant positive association between “herbicide exposure” and NHL (OR = 1.72; 95% CI: 1.18-2.51). Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02 (95% CI:

1.10-3.71). The ORs for glyphosate exposure of <10 days and >10 days were 1.69 (95% CI: 0.70-4.07) and 2.36 (1.04-5.37), respectively. The ORs for glyphosate were 1.11 (95% CI: 0.24-5.08) and 2.26 (95% CI: 1.16-4.40) for “latency” periods of 1-10 years and >10 years, respectively. In analyses of glyphosate and type of NHL, statistically significant positive associations were observed for small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) (OR = 3.35; 95% CI: 1.42-7.89) and for “unspecified NHL” (OR = 5.63; 95% CI: 1.44-22.0). Odds ratios for the other types (total B-cell lymphomas, grade I-III follicular lymphoma, diffuse large B-cell lymphoma, other specified B-cell lymphoma, unspecified B-cell lymphoma, and T-cell lymphomas) were above 1.0, but were not statistically significant (i.e., the 95% confidence intervals were relatively wide and included the null value of 1.0).

The authors concluded, “Glyphosate was associated with a statistically significant increased OR for lymphoma in our study, and the result was strengthened by a tendency to dose-response effect...” (p. 1662). The authors suggested that their findings are consistent with results of a previous case-control study (Hardell and Eriksson 1999, ASB2012-11838) and pooled analysis (Hardell et al. 2002, ASB2012-11839) that they conducted. In the case-control study, an OR of 2.3 (95% CI: 0.4-13.0), based on 4 exposed cases and 3 exposed controls, was reported for glyphosate and NHL. In the pooled analysis of two case-control studies, which included data from Hardell and Eriksson (1999, ASB2012-11838), an OR of

3.04 (95% CI: 1.08- 8.52) was reported, based on 8 exposed cases and 8 exposed controls. The authors also cited three studies (De Roos et al. 2003, ASB2012-11606; McDuffie et al. 2001; ASB2011-364, De Roos et al. 2005, ASB2012-11605) by other groups as being consistent with their results in that they “also associate glyphosate with different B-cell malignancies such as lymphomas and myelomas.” It should be noted, however, that the relative risk (RR) reported by De Roos et al. (2005, ASB2012-11605) for the highest versus lowest category of cumulative exposure days of glyphosate and NHL in the prospective Agricultural Health Study was 0.9.

Interpretation Issues

Identification of Cases and Potential Referral Bias. It is noteworthy that the cases in the current analysis were identified from some of the same hospitals as the authors’ prior publication; thus, referral bias may have been an issue. In particular, the researchers approached the patients after diagnosis if the physicians deemed it appropriate. Therefore, if the physicians were concerned that their patient’s NHL was associated with agricultural exposures, they may have suggested participation in the study.

Participation Rates and Potential Selection Bias. The authors report a participation rate of 91% and 92% for cases and controls, respectively; however, these figures are based on completed questionnaires out of those who had previously said they would participate in the study. The number of eligible patients (i.e., prior to physician approval to “approach”) was not reported, so the computation of an exact participation rate is difficult. Based on information provided in the paper, participation among cases is estimated to be about 80%. Nonparticipation is a concern for several reasons. First, in a case-control study, an odds ratio will be an accurate representation of the exposure-disease association when the cases are representative of all cases and the controls are representative of the exposure experience of the population that gave rise to the cases. If the final study sample is not representative of this “target population” then measures of effect (e.g., the odds ratio) may not be valid. In addition, one must be concerned about selection bias. Selection bias occurs in a case-control study when the exposure distribution for cases and controls differ for those who participate in the study compared to those who are eligible but do not participate in the study. It is not possible to determine whether there is selection bias without information about nonparticipants.

Strengths and Limitations of Using Living Cases Only versus All Cases (Living + Dead).

The authors noted that 88 potential cases died before they could be interviewed and were therefore excluded from the study. It is also stated in the Discussion that restricting the study to living cases and controls was an “advantage” of the study, as interviewing cases and controls directly compared to interviewing next-of-kin was preferable. While it is generally true that this would be an advantage, the following statement by the authors, therefore, is not accurate, “The study covered all new cases of NHL during a specified time” (p. 1660). The study did not include all new cases; it included only those cases who survived until the time of the interview. Thus, while there may have been an advantage to restricting the study to living cases, there was a trade-off in that the study population did not represent all cases, specifically those cases with more aggressive disease. This disadvantage was not discussed by the authors, nor was the potential bias that could have resulted from excluding many eligible cases.

Exposure Measurement and Information Bias. Exposure was ascertained via a questionnaire oriented towards pesticide and other chemical agents. In addition, interviewers collected information by telephone if “important” data were lacking, incomplete, or unclear. It is

unknown what is meant by “important,” and the proportion of cases and controls who received phone calls was not reported. Thus, information bias may be a concern. Even though interviewers were blinded to case and/or control status, they may have been able to determine this information during the course of the interview. Furthermore, recall bias may be an issue because exposure information was based on participant response and cases and controls may recall and/or report past pesticide exposures differently. No exposure validation techniques were implemented, nor did an industrial hygienist (or any other type of personnel trained in assessing occupational exposures) independently validate/estimate the frequency and/or intensity of exposure. The authors assumed that “some misclassification regarding quantity of exposure has probably occurred, but such misclassification would most probably be nondependent of case/control status, and therefore only weaken any true risks” (p. 1660). They do not provide any explanation as to why they believe that exposure misclassification would be “most probably” nondifferential. If NHL cases believe that pesticides may be related to their disease, then it is certainly possible that they may recall and/or report pesticide exposure differently than NHL-free controls, which could result in odds ratios that are inflated as a result of bias.

Interpretation of “dose-response” analyses. The referent group in the statistical analyses consisted of participants who were unexposed to all pesticides. The dose-response analyses were based on a dichotomy of the median number of days exposed to a particular agent. It is difficult to analyze “dose-response” when only two exposure categories are considered. Furthermore, the dose-response analyses were based on median values of exposure but heterogeneity of cut-points is evident across agents. For example, glyphosate was analyzed as < 10 days and > 10 days, whereas, “other” herbicides were analyzed as < 32 days and > 32 days. Although analytical cut-points were data driven, interpretation across the wide variety of exposures is complicated by the variability in exposure cut-points. In addition, even though the OR for the higher category of exposure days was greater than the OR for the lower category, the two 95% confidence intervals were wide and overlapped considerably (0.70-4.07 and 1.04-5.37).

Thus, it is not clear whether the two point estimates reported (1.69 and 2.36) are significantly different from each other. Finally, this result cited in the “dose-response” analyses may have been confounded by exposure to other herbicides. In Table II (Eriksson et al. 2008, ASB2012-11614), the authors observed elevated associations for other herbicides, including MCPA, 2,4,5-T and/or 2,4-D. The correlation between exposure to glyphosate and other herbicides was not provided nor were analyses of glyphosate-exposed individuals after accounting for the collinear relation between this agent and other agents. The odds ratio for “ever” exposure to glyphosate was attenuated after additional adjustment for other pesticides (Table VII, Eriksson et al. 2008, ASB2012-11614), but multi-variate -adjusted estimates for the “dose-response” odds ratios were not reported.

Unusual Pattern of Positive Associations. The authors conducted multiple comparisons, and one would expect a certain proportion of their findings to be statistically significant (whether in the positive or inverse direction) simply as a result of chance. It is somewhat surprising, therefore, that the vast majority of the ORs presented in this manuscript are greater than 1.0, regardless of the statistical significance. The authors do note that for some of the analyses (e.g., latency), only chemicals for which ORs were greater than 1.5 and for which there were at least 10 exposed cases, or for which there was a statistically significant OR were evaluated. On the other hand, dose-response was evaluated based on the number of exposed subjects and not on the strength or significance of the findings. The authors do not address this directly, but do state in their Discussion, “...several pesticides are chemically related and may exert their effects on humans through a similar mechanism of action, which may explain the wide

range of pesticides that have been related to NHL over time in different countries and with different exposure conditions” (p. 1661). On the other hand, this pattern of positive findings could be a result of bias, including recall bias (or other information bias), selection bias, uncontrolled confounding, or a combination of these and other factors.

Interpretation of Eriksson et al. (2008, ASB2012-11614) in Context of Other Studies. Despite the statement by the authors that, “Recent findings from other groups also associate glyphosate with different B-cell malignancies such as lymphomas and myeloma” (p. 1662), most multi-variate analyses of glyphosate and NHL do not report statistically significant associations (De Roos et al. 2005, ASB2012-11605; De Roos et al. 2003; ASB2012-11606, Hardell and Eriksson 1999, ASB2012-11838; Hardell et al. 2002; ASB2012-11839, Lee et al. 2004; ASB2012-11883, McDuffie et al. 2001; ASB2011-364, Nordström et al. 1998, TOX1999-687) (Tables B.6.5-62 and B.6.5-63). It is notable that Hardell et al. (2002, ASB2012-11839) reported a significant positive association between glyphosate association and NHL, but the multi-variate -adjusted odds ratio was attenuated and not statistically significant. Similar findings were reported by Eriksson et al. (2008, ASB2012-11614). Specifically, the association reported by the authors in the abstract (OR = 2.02; 95% CI: 1.10-3.71) was adjusted for age, sex and year of diagnosis or enrollment. When other pesticides were added to that model (i.e., agents with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subjects), the adjusted odds ratio was 1.51 (95% CI: 0.77-2.94). Thus, the authors’ final statement, “Furthermore, our earlier indication of an association between glyphosate and NHL has been considerably strengthened” is questionable. Their previous findings showed a non-significant association after multi-variate adjustment (OR = 1.85; 95% CI: 0.55-6.20). The 2008 study similarly reported a statistically non-significant association between glyphosate and NHL after multi-variate adjustment (OR = 1.51; 95% CI: 0.77-2.94). The results reported for analyses of duration of exposure and latency of exposure did not adjust for other pesticides, and one would expect that those ORs would also be attenuated.

Summary of Findings: Cohort and Case-Control Studies of Exposure to Glyphosate and Non-Hodgkin Lymphoma

Table B.6.5-62: Cohort Studies

Author Year	Description	No. of Exposed Cases	Type of Relative Risk Estimate	Relative Risk Estimate	95% Confidence Limits	Variables Included in Statistical Model
De Roos et al. 2005 (ASB2012-11605)	57-2,678 vs. 1-20 Cumulative Exposure Days ^a	17	RR	0.9	0.5-1.6	Age at enrollment, education, pack-years of cigarette smoking, alcohol consumption in the past year, family history of cancer in first-degree relatives, and state of residence
	337.2-18,241 vs. 0.1-79.5 Intensity-Weighted Exposure Days ^b	22	RR	0.8	0.5-1.4	Also adjusted for other pesticides

^a Years of use x days per year; categorized by tertiles

^b Years of use x days/year x estimated intensity level; categorized by tertiles

Table B.6.5-63: Case Control Studies

Author Year	Exposure Evaluated	Subgroup Description	No. of Exposed Cases	No. of Exposed Controls	OR	95% CI	Variables Included in Statistical Model
De Roos et al. 2003 (ASB201 2-11606)	Ever exposure to specific pesticide; men only (all 47 pesticides were regressed simultaneously)	Glyphosate (Logistic Regression)	36	61	2.1	1.1-4.0	Age, study site and other pesticides
		Glyphosate (Hierarchical Regression)	36	61	1.6	0.9-2.8	Second-level model incorporated what was known about each true effect parameter prior to seeing the study data
Hardell and Eriksson 1999 (ASB201 2-11838)	Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)	Glyphosate (conditional logistic regression; uni-variate analysis)	4	3	2.3	0.4-13	Age and country (matching factors)
		Glyphosate (conditional logistic regression; multi-variate analysis)	4	3	5.8	0.6-54	Multi-variate variables not listed by authors
Hardell et al. 2002 (ASB201 2-11839)	Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)	Glyphosate (conditional logistic regression; uni-variate analysis)	8	8	3.04	1.08-8.52	Age and county (matching factors); study, study area (county), and vital status
		Glyphosate (conditional logistic regression; multi-variate analysis)	8	8	1.85	0.55-6.20	Multi-variate variables not listed by authors
Lee et al. 2004 (ASB201 2-11883)	Exposure to individual pesticides	Glyphosate use, Non-asthmatics	53	91	1.4	0.98-2.1	Age, state, vital status
		Glyphosate use, Asthmatics	6	12	1.2	0.4-3.3	
McDuffie et al. 2001 (ASB201 1-364)	Exposure to individual active chemicals	Glyphosate (Round-Up)	51	133	1.26	0.87-1.80	Strata for age and province of residence
		Glyphosate (Round-Up)	NR	NR	1.20	0.83-1.74	Plus statistically significant medical variables

Author Year	Exposure Evaluated	Subgroup Description	No. of Exposed Cases	No. of Exposed Controls	OR	95% CI	Variables Included in Statistical Model
Nordström et al. 1998 (TOX199 9-687)	Exposure to specific herbicides, insecticides, and fungicides	Glyphosate	4	5	3.1	0.8-12	Age and country (matching factors)
Eriksson et al. 2008 (ASB201 2-11614)	Exposure to specific herbicides regardless if they also had been exposed to phenoxyacetic acids or not	Glyphosate	29	18	2.02	1.10-3.71	Age, sex, and year of diagnosis or enrollment
			29	18	1.51	0.77-2.94	Age, sex, and year of diagnosis or enrollment and pesticides with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subject
	Exposure to herbicide stratified by median number of days among exposed controls	Glyphosate ≤ 10 days	12	9	1.69	0.70-4.07	Age, sex, and year of diagnosis or enrollment
		Glyphosate >10 days	19	9	2.36	1.04-5.37	
	Exposure to specific herbicides according to different lymphoma entities	Glyphosate: B-Cell lymphomas	NR	NR	1.87	0.998-3.51	Age, sex, and year of diagnosis or enrollment
		Lymphocytic lymphoma/B-CLL	NR	NR	3.35	1.42-7.89	
		Follicular grade I-II	NR	NR	1.89	0.62-5.79	
		Diffuse large B-cell Lymphoma	NR	NR	1.22	0.44-3.35	
		Other specified B-cell lymphoma	NR	NR	1.63	0.53-4.96	
		Unspecified B-cell Lymphoma	NR	NR	1.47	0.33-6.61	
		T-cell lymphomas	NR	NR	2.29	0.51-10.4	
		Unspecified NHL	NR	NR	5.63	1.44-22.0	

Author(s)	Year	Study title
George, J. Prasad, S. Mahmood, Z. Shukla, Y.	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach Journal of Proteomics Volume: 73, Pages: 951-964 ASB2012-11829

Abstract*

Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF-1 alpha chain, carbonic anhydrase III, annexin II, calyculin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu-Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc. The up-regulation of calyculin, calgranulin-B and down-regulation of superoxide dismutase [Cu-Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.

* Quoted from article

Klimisch evaluation

Reliability of study:

Reliable with restrictions

Comment:

Non-guideline mechanistic study. Scientifically acceptable study with deficiencies (controls with glyphosate alone, and co-formulants were not included)

Relevance of study:

Relevant with restrictions (Glyphosate formulation not glyphosate alone was tested.)

Klimisch code:

2

Additional comments:

The authors use glyphosate as a synonym for what is really a glyphosate based formulated product. Doses in this study are not representative of human exposures to glyphosate or glyphosate based formulations. Mice in the tumor promoting group VIII received topical applications of concentrated glyphosate formulated product three times per week for over thirty weeks without washing after an initial treatment with the potent tumor initiator DMBA. Glyphosate had been shown to have very low dermal absorption, even in formulated products, and since is non-volatile, would likely accumulate on mouse skin. Surfactants are typically irritating and non-volatile. Given the irritation potential of the unwashed exposed mouse skin over the course of thirty or more weeks, tumor promotion may be a physical response to substantial localized dermal irritation. Epidemiological studies reported above note no association with glyphosate and either skin or lip cancers.

Label directions outline appropriate personal protective equipment such as gloves and long sleeves. Furthermore, any dermal exposure of concentrated product to human skin would prove irritating and prompt handlers to wash off soon after dermal exposure.

Human *in vitro* dermal absorption studies reported for a range of glyphosate based formulations containing different surfactant systems all demonstrate extremely low dermal absorption of glyphosate active ingredient for concentrated products, of less than 0.2 %. Test material recovery in each of the four reported dermal absorption studies was very good, close to 100 %. Most of the glyphosate was removed during skin surface washing at either eight or twenty four hours of *in vitro* human skin exposure. This also suggests significant potential for accumulation of glyphosate on the surface of the mice skin in George et al. (2010, ASB2012-11829).

The up-regulation / down-regulation of protein expression reported after a single dermal dose of a glyphosate formulated product (proteomics experiment, group II), while interesting, does not demonstrate any toxicological endpoint. Rather, perturbations may well represent normal homeostatic fluctuations and be a natural response to insult.

Author(s)	Year	Study title
Seralini, 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. Food and Chemical Toxicology 50, 4221-4231 ASB2012-15514

Abstract*

The health effects of a Roundup-tolerant genetically modified maize (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water), were studied 2 years in rats. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls, the pituitary was the second most disabled organ; the sex hormonal balance was modified by GMO and Roundup treatments. In treated males, liver congestions and necrosis were 2.5–5.5 times higher. This pathology was confirmed by optic and transmission electron microscopy. Marked and severe kidney nephropathies were also generally 1.3–2.3 greater. Males presented 4 times more large palpable tumors than controls which occurred up to 600 days earlier. Biochemistry data confirmed very significant kidney chronic deficiencies; for all treatments and both sexes, 76% of the altered parameters were kidney related. These results can be explained by the non linear endocrine-disrupting effects of Roundup, but also by the overexpression of the transgene in the GMO and its metabolic consequences.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	The study was performed to investigate the long term toxicity and carcinogenicity. However the study design does not agree with the OECD guidelines on long term toxicity and carcinogenicity.
Relevance of study:	Relevant with restrictions (Glyphosate formulation not glyphosate alone was tested.)
Klimisch code:	3

Comments:

Seralini et al. (2012, ASB2012-15514) submitted a report of long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. The health effects have been studied 2 years in rats. Six groups of rats were fed with 11, 22 and 22 % of genetically modified NK603 maize either treated or not with Roundup. Three further groups of rats were fed with control diet and had access to water supplemented with 50 ng/L, 400 mg/L and 2.25 g/L of the commercial product Roundup (GT Plus, 450 g/L of glyphosate). The pure active substance glyphosate was not tested in this study.

The study is not considered reliable because of several important limitations. According to the authors the studies have been performed to investigate the long term toxicity and carcinogenicity. However, the number of animals per dose and sex was only 10 and also the further study design does not agree with the OECD guidelines on long term toxicity and carcinogenicity. The spontaneous incidence of mammary tumors in the used Sprague Dawley rats is much higher than in most other rat strains. Therefore, a higher number of animals would be necessary for the differentiation between treatment related carcinogenicity and accidental aberrations. Also for the assessment of mortality and further described toxic effects a higher number of animals would be needed.

The presented results in the publication are incomplete and therefore, an evaluation of the presented results was complicated.

The study was extensively discussed and criticized in the public. In an additional paper Seralini et al. (2013, ASB2013-10985) gave some answers to the critics. The authors admit that the study “should not be considered as a final point in knowing the toxicological effects of NK603 and Roundup” and that the study has limits.

Author(s)	Year	Study title
Chruscielska, K. Brzezinski, J. Kita, K. Kalhorn, D. Kita, I. Graffstein, B. Korzeniowski, P.	2000	Glyphosate Evaluation of chronic activity and possible far-reaching effects. Part 1. Studies on chronic toxicity Pestycydy 2000, (3-4), 11-20 ASB2013-9829

Abstract*:

The combined test of chronic toxicity and carcinogenicity of glyphosate was performed on Wistar-RIZ rats. The herbicide was administered in water at concentrations: 0, 300, 900, 2700 mg/L. The examination of the peripheral blood parameters and the smears of bone marrow did not reveal harmful effect of the herbicide on haematopoietic system of rats. The biochemical parameters determined on blood and urine only in some cases showed significant deviations in comparison with the control group, but in any examined indices dose-effect-time occurred what could manifest the toxic influence of glyphosate. In pathomorphological studies on the organs no correlation was stated between the number of observed tumours and the concentrations of the herbicide. It indicates lack of pathogenic influence of glyphosate on neoplastic pathogenesis.

* Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	The published details of the study are limited. However, according to the authors the study was performed on basis of OECD guideline No. 453
Relevance of study:	Relevant
Klimisch code:	2

Comments:

The active substance glyphosate was used in the study and the study was performed on basis of OECD guideline 453. The number of animals per dose group and sex (85 animals) was even higher than required in guideline 453. Therefore, the study is considered to be relevant. No carcinogenic effects have been registered in the study.

B.6.6 Reproductive toxicity (Annex II A 5.6)

Introduction into this chapter by the RMS

For higher efficiency of the review and for the sake of transparency, the descriptions of methods and study results in the GTF dossier were virtually not amended and even the conclusions were kept as provided. However, each study that is described in detail was commented by RMS. These remarks on bottom of each study description are clearly distinguished from the original submission by a caption and are always written in italics. In addition, redundant parts (in particular the so-called "executive summaries") have been deleted and the structure of the original submission was significantly changed to make it more transparent and comprehensible.

The overall assessment of reproductive toxicity of glyphosate by the RMS is provided in Volume I (2.6.6) of the present RAR.

Comments by the GTF on the first draft of the RAR (July 1013) have been partly included in the present report. Responses by RMS to GTF are written in italics and given below. This approach was taken to avoid doubling of comments/responses at a later timepoint.

B.6.6.1 Two generation reproductive toxicity in the rat

The reproductive toxicity of glyphosate was tested in a variety of multi-generation studies in rats. For the previous EU evaluation, a total of 8 studies in rats had been submitted of which four were still considered acceptable or, in case of a single one-generation study, at least supplementary upon re-evaluation. The studies by [REDACTED] (1981, TOX9552385), by [REDACTED] (1985, TOX9650161) and by [REDACTED] (both 1988, TOX9551832 and TOX9551965), however, were deleted from current evaluation due to major deficiencies and/or because the dose levels were much too low and therefore one could not expect the occurrence of any toxic effects.

Three new studies were provided in the GTF dossier and were submitted either for the first time for this evaluation or had been subject to JMPR evaluation (JMPR, 2004, ASB2008-6266) yet.

Reference: IIA, 5.6.1/01

Report: [REDACTED] (2007)

Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat

Data owner: Nufarm

SPL project no.: 2060/0013

Date: 2007-10-31 (amended 2008-04-08 and 2008-08-08)

not published

ASB2012-11494

Guidelines: OECD 416 (2001), JMAFF 2-1-17 (2001), US-EPA OPPTS 870.3800 (1998)

Deviations: None

GLP: yes

Acceptability: See RMS comment

Dates of experimental work: 2005-11-18 to 2006-11-06

Materials and methods

Test material:

Identification: Glyphosate technical

Description: White crystalline solid

Lot/Batch #: H05H016A

Purity: 95.7 % (w/w)

Stability of test compound: Not reported

Vehicle and/or positive control: Plain diet

Test animals:

Species: Rat

Strain: Sprague-Dawley CrI:CD (SD) IGS BR

Source: [REDACTED]

Age: Approximately 8 weeks

Sex:	Males and females
Weight at dosing:	Males: 138 – 257 g; females: 140 – 195 g
Acclimation period:	At least 14 days
Diet/Food:	Rodent PMI 5002 (certified) diet (BCM IPS Limited, UK), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	Initially in groups of up to four in polypropylene cages with stainless steel grid floors and tops, suspended over polypropylene trays lined with absorbent paper. During mating animals were housed one male : one female. Mated females were housed individually during gestation and lactation in polypropylene cages with solid floors and stainless steel lids, furnished with softwood flakes.
Environmental conditions:	Temperature: $21 \pm 2^{\circ}\text{C}$ Humidity: $55 \pm 15\%$ Air changes: at least 15/hour 12 hours light/dark cycle

Study design and methods:

In life dates: 2005-11-18 to 2006-11-06

Animal assignment and treatment:

In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females) glyphosate technical in diet. The dose levels were chosen based on results of a previously conducted study. After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. At weaning of offspring from the F0 mating phase, groups of twenty-four male and twenty-four female offspring from each dose group were selected to form the F1 generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 *post partum*, followed by the termination of all F0 male dose groups. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups.

Diet preparation and analyses

For preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet at a constant speed for 19 minutes in a Hobart QE200 mixer. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes in a Hobart H800 mixer.

The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration weekly for the first four weeks of the study and monthly thereafter.

Clinical observations

A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.

Body weight

Individual body weights were recorded for F0 males on Day 1 (prior to treatment) and at weekly intervals for F0 and F1 males until termination. F0 and F1 females were weighed daily until mating was evident. Bodyweights for females showing evidence of mating were recorded on Days 0, 7, 14 and 21 *post coitum*. Females with live litters were weighed on Days 1, 4, 7, 14 and 21 *post partum*.

Food consumption and compound intake

During the maturation period, weekly food consumption was recorded for each cage of adults. For females showing evidence of mating, food consumption was recorded for the periods covering Days 0 - 7, 7 - 14 and 14 - 21 *post coitum*. For females with live litters, food consumption was recorded for the period covering Days 1 - 4, 4 - 7, 7 - 14, 14 - 21 *post partum*.

Food conversion efficiency (the ratio of bodyweight change / dietary intake) was calculated retrospectively for males for both the pre-mating and post-mating phases of the study. For females, food conversion efficiency was only calculated for the pre-mating phases of the study. Due to offspring growth, milk production and weaning, food efficiency could not be accurately calculated for the gestation and lactation phases of the study.

Water consumption

Water intake was observed daily by visual inspection of water bottles for any overt change.

Reproduction parameters

Oestrus cycle

Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous.

Pregnancy and parturition

Pregnant females were observed at approximately 0830, 1230 and 1630 hours daily, and at approximately 0830 and 1230 hours on weekends and public holidays. In addition, the females were observed around the period of expected parturition. The date of mating, date and time of start and end of parturition and duration of gestation was recorded.

Litter data

The following litter data were recorded:

The number of offspring born, the number of offspring alive recorded daily and reported on Day 1, 4, 7, 14, 21 *post partum*. On Days 1, 4 and 21, the sex of individual offspring was recorded. The clinical condition of offspring during lactation, as well as individual offspring and total litter weights were recorded after birth on Day 1, 4, 7, 14.

Physical and sexual development

All live offspring were observed for the detachment and unfolding of pinna, incisor eruption and eyelid separation and assessed for reflexological response to stimuli by assessing surface righting reflex on Day 1 *post partum* and air righting reflex on Day 17 *post partum*. Pupillary reflex and auditory startle response were performed on Day 21 *post partum*.

All selected F1 offspring were observed for sexual development and the bodyweight for each individual animal at the time of sexual maturation was recorded. In addition, the ano-genital distance was recorded for all F2 generation offspring on day 1 *post partum*.

Sacrifice and pathology

All surviving adult females and surviving offspring, except offspring selected to form the F1 generation, as well as surviving males were sacrificed on Day 21 post partum.

All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For females the uterine implantation sites were counted. In addition, the corpora lutea of all ovaries from pregnant females were counted at necropsy.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the study sampled, weighed and preserved, except for the thyroids, which were weight after fixation:

adrenals, brain, left cauda epididymis, epididymides, kidneys, liver, ovaries, prostate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, thymus, thyroid glands, and uterus (with cervix and oviducts).

The following organs from one male and one female offspring from the F0 and F1 pairings were weighed: brain, spleen, thymus, and uterus.

The following tissues were preserved from all F0 males and females from each dose group in 10 % buffered formalin, except for the right epididymis, right testis, which were fixed in Bouins fluid and 70% IMS: adrenals, coagulating gland, right epididymis, ovaries, right testis, pituitary, prostate, seminal vesicles, Uterus (with oviducts) and cervix, vagina and all gross lesions.

A detailed histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on animals that died or were killed in extremis.

During the histopathological examination there were indications of treatment-related changes in the adrenal glands for the F1 animals. Thus, the microscopic examination was subsequently extended to include similarly prepared sections of adrenals from the F1 animals from the 5000 and 1500 ppm dose groups.

Semen assessment

At necropsy of adult F0 and F1 males at least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the testis and cauda epididymis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number

From ten control and ten high dose females of the F1 generation slides of the ovaries were prepared and analysed for visible oocytes. The identified oocytes were classified as small, medium or large follicles.

Statistics

Organ weight (absolute and relative to terminal bodyweight), weekly bodyweight gain, litter weights and offspring bodyweights were assessed for dose response relationships by linear regression analysis, followed by one way analysis of variance (ANOVA) incorporating Levene's test for homogeneity of variance. Where variances were shown to be homogenous, pairwise comparisons were conducted using Dennett's test. Where Levene's test showed unequal variances the data were analysed using non-parametric methods: Kruskal-Wallis ANOVA and Mann-Whitney 'U' test.

The non-parametric methods were also used to analyse implantation loss, offspring sex ratio and developmental landmarks and reflexological responses.

Probability values (p) are presented as follows:

$p < 0.001$ ***

$p < 0.01$ **

$p < 0.05$ *

$p \geq 0.05$ (not significant)

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:

1. Chi-squared analysis for differences in the incidence of lesions occurring with an overall frequency of one or greater.

2. Kruskal-Wallis one-way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

Probability values (p) were calculated as follows:

$p < 0.001$ +++ --- ***

$p < 0.01$ ++ -- **

$p < 0.05$ + - *

$p < 0.1$ (+) (-) (*)

$p \geq 0.1$ N.S. (not significant)

(+)-signs indicate positive differences from the control group, and (-)-signs indicate negative differences. * refer to overall differences between group variation which is non-directional.

Results and discussion

Analysis of dose formulations

Stability analyses indicated that the dose preparations at nominal concentrations of 1500, 5000 and 15000 ppm were stable for at least six weeks at ambient temperature.

Analyses for homogeneity at the start of treatment indicated that the dose preparations were homogeneous.

Analyses for achieved concentration performed on ten separate occasions demonstrated that the prepared dietary admixture concentrations given to the animals were in the range of 83 to 102% of the nominal concentration.

Test compound intake

The group mean achieved dosages are summarised in Table B.6.6-1 below.

Table B.6.6-1: Group mean achieved dose levels

Group	Dietary concentration	Estimated dose level	Mean achieved dose level			
	(ppm)	(mg/kg bw/day)	(mg/kg bw/day)			
			Males	Females		
				Maturation	Gestation	Lactation
Control	0	0	0	0	0	0
Low	1500	75	104	126	108	252
Intermediate	5000	250	351	423	358	808
High	15000	750	1063	1273	1109	2520

Mortality

There were no test substance related mortalities.

Four unscheduled deaths occurred during the study. In the F0 generation one male of the low dose group and one female of the mid dose group was killed on humane reasons on Days 87 and 103, respectively. The male exhibited a mass of about 3 x 4 cm on the lower jar. The female was in extremis following a suspected prolonged parturition. One high dose female was found dead on Day 97 possibly due to complications during parturition.

In the F1 generation one control female was killed on Day 99 following severe clinical signs (pallor of the extremities, lethargy, pilo-erection, hunched posture and staining around the ano-genital region); however the aetiology of the signs was not established.

Clinical observations

No treatment-related clinical signs of toxicity were noted. Clinical signs observed in control and treated animals of the F0 and F1 generation are summarised in Table B.6.6-2 and Table B.6.6-3 below. These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.

Table B.6.6-2: Observed clinical signs in F0 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (1500 ppm)		Mid (5000 ppm)		High (15000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
Abrasion to dorsal region	2/28	0/28	0/28	0/28	0/28	0/28	1/28	0/28
Generalised fur loss	5/28	5/28	3/28	5/28	2/28	6/28	2/28	3/28
Red/brown staining around snout	4/28	0/28	4/28	0/28	1/28	3/28	5/28	0/28
Red/brown staining of fur	1/28	0/28	1/28	0/28	2/28	2/28	1/28	0/28
Red/brown staining around eyes	1/28	0/28	1/28	1/28	0/28	0/28	3/28	0/28
Swollen face (due to overgrowth tooth)	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28
Cranial abrasion	0/28	0/28	0/28	0/28	0/28	0/28	2/28	0/28
Red stained urine	0/28	0/28	0/28	0/28	0/28	0/28	1/28	0/28
Facial scab formation	1/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Scab formation	1/28	0/28	1/28	0/28	1/28	0/28	0/28	0/28
Large mass under lower jar	0/28	0/28	1/28	0/28	0/28	0/28	0/28	0/28
Mass on dorsal region	0/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Scab formation around right eye	0/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Physical injury to tail apex	0/28	0/28	0/28	1/28	0/28	0/28	0/28	0/28
Stained fur on head	0/28	0/28	0/28	0/28	0/28	0/28	0/28	1/28
Red swollen ears	0/28	1/28	0/28	0/28	0/28	1/28	0/28	1/28
Blood seen without evidence of offspring born	0/28	1/28	0/28	0/28	0/28	0/28	0/28	0/28
Blood around vagina (suspected prolonged parturition, killed in extremis)	0/28	0/28	0/28	0/28	0/28	1/28	0/28	0/28
Pilo-erection	0/28	0/28	0/28	0/28	0/28	0/28	0/28	1/28
Exophthalmia	0/28	1/28	0/28	0/28	0/28	0/28	0/28	1/28

* x/y: number affected / total number of animals in group

Table B.6.6-3: Observed clinical signs in F1 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (1500 ppm)		Mid (5000ppm)		High (15000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
Generalised fur loss	3/28	4/28	0/28	2/28	0/28	6/28	0/28	4/28
Red/brown staining around eyes	2/28	1/28	0/28	0/28	1/28	0/28	0/28	0/28
Red/brown staining of fur	0/28	1/28	2/28	0/28	0/28	2/28	1/28	1/28
Red/brown staining around snout	1/28	7/28	1/28	0/28	4/28	7/28	1/28	4/28
Scabbing and fur loss around eye	0/28	/28	0/28	/28	0/28	/28	1/28	/28
Protruding sternum	0/28	2/28	0/28	3/28	0/28	3/28	0/28	0/28
Lethargy	0/28	1/28	0/28	0/28	0/28	0/28	0/28	0/28
Hunched posture	0/28	1/28	0/28	0/28	0/28	0/28	0/28	0/28
Staining around ano-genital region	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28
Pallor of extremities	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28

* x/y: number affected / total number of animals in group

Body weight

No adverse effect of body weight change was evident for treated animals in comparison to controls throughout the treatment period for both the F0 and F1 generations except for *post-partum* females treated with 15000 ppm (see Table B.6.6-4). During the final week of lactation, both the F0 and F1 generations showed statistically significant less body weight loss in comparison to controls ($p < 0.001$ and $p < 0.01$ respectively).

Table B.6.6-4: Body weight changes during lactation (Group mean values)

Dietary concentration (ppm)	No. of animals		Body weight Change (g) at Day			
			4	7	14	21
F0 Generation						
0 (Control)	26	mean	15	22	0	-23
		sd	14	9	15	10
1500	27	mean	16	16	3	-26
		sd	9	9	13	13
5000	26	mean	16	18	1	-23
		sd	14	13	11	11
15000	26	mean	18	18	1	-8***
		sd	11	12	14	14
F1 Generation						
0 (Control)	26	mean	14	9	9	-16
		sd	11	13	14	13
1500	27	mean	14	16	3	-21
		sd	7	11	9	17
5000	26	mean	17	10	5	-17
		sd	12	10	13	13
15000	26	mean	16	11	10	-4**
		sd	9	9	12	13

sd - standard deviation

** - significantly different from control group $p < 0.01$

*** - significantly different from control group $p < 0.001$

Water consumption

Daily visual inspection of water bottles showed no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations, when compared to their concurrent controls.

Reproductive parameters

Oestrus cycle

There were no toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation

There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

Litter data

Size and Viability

No overt differences in litter size and viability were detected. The mean numbers of corpora lutea and subsequent number of implantations did not indicate any adverse effect of dietary exposure and pre and post implantation loss for treated animals were essentially similar to controls. There were no toxicologically significant differences in sex ratio for both F0 - F1 and F1 - F2 litters.

Growth and Development

No adverse effects on mean offspring bodyweights, bodyweight change or development were detected for male and female offspring in comparison to their controls.

Clinical signs

No clinically observable signs of toxicity were observed for offspring from treated animals.

Pathology

Necropsy

There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1 animals, nor in the offspring.

Organ weights

F0 females treated with 15000 ppm displayed statistically significant increases in liver weights, both absolute and relative to terminal body weight ($p < 0.001$). An increase in liver weights was also noted for F1 females treated with 15000 ppm (absolute: $p < 0.05$, relative: $p < 0.01$). In the absence of any histopathological changes in the liver, and as increased liver weights without histopathological changes were also noted in another repeated dose toxicity study this finding is considered as an adaptive response rather than an adverse effect. Furthermore, F0 females treated with 15000 ppm displayed an increase in kidney weights, both absolute ($p < 0.001$) and relative to terminal body weight ($p < 0.01$) (see Table B.6.6-5). No such observations were detected for males treated with 15000 ppm from either generation.

Table B.6.6-5: Liver and kidney weights (relative and absolute) of females (Group mean values)

Dietary concentration (ppm)	No. of animals		Organ weight (g)			
			Liver		Kidney	
			Absolute	Relative	Absolute	Relative
			F0 Generation			
0 (Control)	26	mean	15.0328	4.3103	2.4315	0.6977
		sd	1.0493	0.2864	0.1706	0.0548
1500	27	mean	15.1465	4.3027	2.5395	0.7233
		sd	1.4948	0.3435	0.1602	0.0560
5000	27	mean	15.8791	4.3570	2.5654*	0.7062
		sd	1.7649	0.2810	0.2361	0.0592
15000	26	mean	16.9704***	4.6806***	2.7096***	0.7490**
		sd	1.7620	0.2977	0.2203	0.0521
			F1 Generation			
0 (Control)	22	mean	16.4887	4.5970	2.6792	0.7483
		sd	2.0275	0.4038	0.4137	0.1070
1500	23	mean	16.3848	4.6047	2.5777	0.7257
		sd	1.7744	0.2858	0.2776	0.0647
5000	24	mean	17.2591	4.6543	2.8124	0.7585
		sd	2.0969	0.3628	0.5326	0.1229
15000	23	mean	18.0724*	4.9591**	2.7660	0.7578
		sd	1.2434	0.3130	0.2616	0.0517

sd - standard deviation

* - significantly different from control group $p < 0.05$ ** - significantly different from control group $p < 0.01$ *** - significantly different from control group $p < 0.001$

There were no toxicologically significant intergroup differences detected for the brain, spleen or thymus for offspring of either sex from either generation. Furthermore, there were no differences in uterus weights for treated females from either generation when compared to controls.

Sperm assessment

There were no toxicologically significant effects on the concentration, motility or morphology of samples of sperm from treated F0 and F1 generation males when compared to their controls. Furthermore, no abnormal sperm were detected in the control and treated males from either generation.

Oocyte assessment

There were no toxicologically significant differences in follicle numbers for F1 females treated with 15000 ppm when compared to controls.

Histopathology

No treatment-related changes were detected in the F0 generation animals.

In the F1 generation cortical vacuolation of the adrenal glands was observed with a lower incidence and with generally lower grades of severity among males treated with 15000 ppm ($p < 0.05$), 5000 ppm ($p < 0.05 - 0.01$), and 1500 ppm ($p < 0.1 - 0.05$) when compared to controls. The group distribution of incidence and of severity grades may also suggest a consequence of treatment. However, the absence of a dose-related response, may suggest that a higher than normal background incidence of the condition among control male rats may have contributed to the effect on this occasion.

Table B.6.6-6: Incidence of adrenal cortical vacuolation in males at terminal kill

	Historical control data	Dietary concentration (ppm)							
		0		1500		5000		15000	
Generation	--	F0	F1	F0	F1	F0	F1	F0	F1
Animals examined	234	28	24	27	24	28	24	28	24
adrenal cortical vacuolation									
Absent	153	20	7	--	14	--	16	16	14
Present	81	8	17	--	10*	--	8**	12	10***
Minimal	57	6	10	--	6	--	6	8	7
Slight	23	2	7	--	4	--	2	4	2
moderate	1	0	0	--	0	--	0	0	1
% present	34.6	28.6	71%	--	24	--	33	42.9	42

* - significantly different from control group $p < 0.1$ – $p < 0.05$

** - significantly different from control group $p < 0.01$ – $p < 0.05$

*** - significantly different from control group $p < 0.05$

All remaining morphological changes were those commonly observed in laboratory maintained rats of the age and strain employed and, since there were no differences in incidence or severity between control and treatment groups, all were considered to be without toxicological significance.

Conclusion by the Notifiers

The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 15000 ppm for two successive generations resulted in possible treatment-related changes at 15000 ppm. The effects however were considered not to represent an adverse health effect, therefore the NOAEL was considered to be 15000 ppm (equivalent to 1063 and 1634 mg/kg bw/day, for males and females, respectively) for adult toxicity for both the F0 and F1 generations.

The NOAEL for reproductive and developmental toxicity, for both generations and offspring was considered to be 15000 ppm.

Comment by RMS:

The study is considered acceptable. The NOAEL for parental, reproductive and offspring toxicity is considered to be 5000 ppm (approx. equivalent to 351 mg/kg bw/d) instead the proposal by the Notifiers being 15000 ppm (> 1000 mg/kg bw/d): At highest dose level of 15000 ppm increased organ weights in liver (F0 & F1 females) and kidneys (F0 females) were observed. The Notifiers stated that there is no toxicological concern regarding the significant increased liver weights due to the absence of any histopathological changes in the liver. In fact, in the present study no evidence for histopathological examination of the liver was given.

*At this high dose level a significant decrease in homogenisation resistant spermatids (HRS, cauda epididymis) was counted in F0 males (Control: 399.9 million/gram; 15000 ppm: 309.0 million/gram**). No remarkable effects were seen at lower dose levels.*

Furthermore, in F1 male offsprings sexual maturation (preputial separation) was delayed at 15000 ppm without any additional developmental retardation (e.g. body weight, please see Table B.6.6-7 below). The authors of the study considered this finding in F1 males (45.9 d versus control 43.0 d) to be unrelated to treatment, because no effects on sexual maturation were evident for females and there were no differences in mating performance. Sperm changes and histopathological examinations did not reveal any changes in the testis or epididymes. Although, the later onset of preputial separation in male offsprings at 15000 ppm

had obviously no impact on reproductive performance in week 29, a treatment related effect on sexual maturation at parental toxic dose cannot be excluded.

All in all, the NOAEL of 5000 ppm (ca. 351 mg/kg bw/d) is considered to be more appropriate regarding parental, reproductive and offspring toxicity.

Table B.6.6-7: Sexual Maturation (balano preputial separation) of F1-males (provided by RMS)

Diet concentration (ppm)	Number of animals		Age (Days) at completion	Body weight (g) at attainment
0 (Control)	24	mean	43.0	210
		sd	2.3	23
1500	24	mean	43.3	216
		sd	1.6	22
5000	24	mean	43.5	219
		sd	2.3	22
15000	24	mean	45.9**	230**
			3.1	28

** $p < 0.01$

Reference:

IIA, 5.6.1/02

Report:

██████████ (2000)

Glyphosate acid: Multigeneration reproduction toxicity study in rats

██

Data owner: Syngenta/Monsanto

Report No.: ██████████/P/6332

Date: 2000-06-16

not published

TOX2000-2000

Guidelines:

OECD 416, Annex V 67/548/EEC, 9. ATP 87/302/EEC OJEC, L133, 47-50 (1988), US-EPA OPPTS 870.3800 (1998)

Deviations:

None

GLP:

Yes

Acceptability:

See RMS comment.

Dates of experimental work: 1998-09-01 to 1999-12-10

Materials and methods

Test material:

Identification:

Glyphosate acid, technical

Description:

White solid

Lot/Batch #:

Y04707/082

Purity:

97.6% (w/w)

Stability of test compound:

At least 10 years at ambient temperature

Vehicle and/
or positive control:

Plain diet

Test animals:

Species:	Rat
Strain:	Alpk:AP _f SD (Wistar-derived)
Source:	[REDACTED]
Age:	At least 5 weeks old
Sex:	Males and females
Weight at dosing:	Males: approx. 160 g; females: approx. 140 g
Acclimation period:	At least 14 days
Diet/Food:	CT1 diet (Special Diet Services Ltd., Witham, Essex, UK), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	Rats were housed in pairs (same sex) in multiple rat racks (with rats of the same group in adjacent cages). During mating animals were housed one male : one female. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were housed in pairs until termination. Males were housed up to four per cage after being used for mating.
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 50 ± 20 % Air changes: at least 15/hour 12 hours light/dark cycle

Study design and methods:

In life dates: not reported

Animal assignment and treatment:

In a two-generation reproduction study groups of 26 Alpk:AP_fSD rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate acid in diet. The dose levels were chosen based on results of a previously conducted chronic toxicity study.

After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. On Day 29 *post partum*, groups of twenty-six male and twenty-six female offspring from each dose group of the F0 generation were selected to form the F1 generation. F0 males were terminated after the completion of littering and females were terminated on or soon after Day 29 of lactation. Unselected offspring were terminated at Day 29 *post partum*. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned off on Day 29 *post partum* and terminated thereafter.

Diet preparation and analyses

For preparation of diet mixtures (60 kg) a known amount of the test substance was mixed with a small amount of basal diet in a mortar using a pestle. Further milled diet was added to give a pre-mix of 1000 g. Each pre-mix was grounded at a constant speed for 15 min with an automatic pestle and mortar. This pre-mix was then added to a larger amount of basal diet and blended for further 6 minutes in a Pharma Matrix Blender Model PMA 150S (TK Fielder). Control diet was treated in the same way but without addition of the test substance. The stability and homogeneity of the test material in diet were determined in the lowest and the

highest dose. Dietary admixtures were analysed for achieved concentration at a 2 month interval.

Clinical observations

A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.

Body weight

Individual body weights were recorded for F0 adults immediately prior to treatment and weekly thereafter throughout the pre-mating period. F0 males were weighed weekly thereafter until termination. Successfully mated F0 females were weighed on Day 1, 5, 8, 15 and 22 of gestation and on Day 1, 5, 8, 15, 22 and 29 *post partum*. Initial body weights for the F1 adults were recorded at selection on Day 29 *post partum* and weekly thereafter throughout the pre-mating period. F1 males were weighed weekly thereafter until termination. Successfully mated F1 females were weighed on Day 1, 5, 8, 15 and 22 of gestation and on Day 1, 5, 8, 15, 22 and 29 *post partum*. All rats were weighed at termination.

Food consumption and compound intake

Food consumption for each cage was recorded throughout the pre-mating period and calculated on a weekly basis. Food utilisation was calculated as the bodyweight gained by the rats in the cage per 100 g of food eaten. Food consumption was also recorded for females during gestation and lactation and calculated on a weekly basis.

Reproduction parameters

Oestrus cycle

Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous. A vaginal smear was also taken and examined from all F0 and F1 females at termination.

Reproductive performance

The success of mating (production of viable litter) was established. Length of gestation was measured in days from the date of the positive smear to the date of birth. Pre-coital interval was measured as the number of days from the date of pairing to the date of the positive smear.

Litter data

The following litter data were recorded:

The number of offspring born and the number of offspring alive were counted within 24 h after parturition and thereafter on Day 5, 8, 15, 22 and 29 *post partum*. The sex and the litter weight was also recorded at these times. Any clinical findings were recorded. Litters were examined for dead or moribund pups at least once daily.

Physical and sexual development

All selected F1 offspring were observed for sexual development and the bodyweight for each individual animal at the time of sexual maturation was recorded.

Sacrifice and pathology

All surviving adult females and surviving offspring, except offspring selected to form the F1 generation, were sacrificed on Day 29 post partum. Males were sacrificed at completion of the littering. All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For F0 and F1 females the uterine implantation sites were counted.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the study were sampled, weighed and preserved:

adrenal gland, brain, left and right epididymides and caudae, kidneys, liver, ovaries, prostate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, uterus (with cervix and oviducts).

The following organs from one male and one female offspring from the F1 pairings were weighed: brain, spleen and thymus.

The following tissues were preserved from all F0 males and females from each dose group in 10% buffered formalin, except for the left epididymis, left testis which were fixed in Bouin's fixative: adrenals, brain, coagulating gland, left epididymis, ovary, left testis, pituitary, prostate, seminal vesicle, uterus (with oviducts) and cervix, vagina and all gross lesions.

Beside all pups killed in extremis (age 18-29 days) 3 male and 3 female per F2-litter were given a macroscopic examination at termination on Day 29 post partum. One of the 3 pups/sex/litter was used for organ weight determination as described above. Following tissues were stored from these pups: brain, spleen, thymus, salivary gland. Abnormal tissue from all these pups were taken and fixed as described earlier.

The reproductive organs from animals suspected of reduced fertility were processed for histopathological examination.

Semen assessment

At necropsy of adult F0 and F1 males sperm were taken from the right distal cauda epididymis. At least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the right testis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number

Primordial and small growing follicles were quantified in the left ovary of all F1 females from the control and high dose groups. Quantification was done using five 5 µm thick sections cut from the central third of each ovary and taken at least 100 µm apart and as evenly spaced as possible.

Statistics

One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: analyses of variance (ANOVA), analyses of covariance, ANOVA followed by analyses of covariance, as well as ANOVA following the double arcsine transformation of Freeman and Tukey (1950), or ANOVA following a square root transformation, or Fisher's Exact Test.

All analyses were carried out in SAS (1996). For Fisher's Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance, with the exception of pup organ weights, allowed for the replicate structure of the study design.

Least-squares means for each group were calculated using the LSMEAN Option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's *t*-test, based on the error mean square in the analysis.

All statistical tests were two sided.

Results and discussion

Analysis of dose formulations

The chemical stability of glyphosate acid in the diet at nominal concentrations of 1,000 and 10,000 ppm was consistent for at least 6 weeks (at room temperature). Homogeneity of the test substance in the dietary mixture was satisfactory, percentage deviations from the overall mean were within 4 %. The mean achieved concentrations of glyphosate acid in the preparations were within 9 % of the nominal concentrations and the overall mean concentrations were within 3 % of the nominal concentrations.

Test compound intake

The group mean achieved dosages are summarised in Table B.6.6-8 below.

Table B.6.6-8: Group mean achieved dose levels F0 and F1-generation

Group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)			
		Males*		Females	
			Maturation	Gestation	Lactation
Control	0	0	0	0	0
Low	1000	108.0 [99.4 / F0] [116.5 / F1]	113.9	90.4	227.9
Intermediate	3000	322.2 [292.6 / F0] [351.8 / F1]	346.8	277.9	752.4
High	10000	1072.9 [984.7 / F0] [1161.0 / F1]	1136.2	910.9	2424.8

* Values of achieved dose levels, separated in F0 and F1 were inserted by the RMS

Mortality

There were no test substance related mortalities.

Seven unscheduled deaths occurred during the study. In the F0 generation one control male was killed for humane reasons during week 9 because it was found to have a ruptured eyeball. In the low level dose group one female was killed for humane reasons during week 14 having failed to litter on time, dead foetuses were present in the uterus. In the intermediate level dose group one female was killed in week 14 on gestation day 23 due to difficulties with parturition. In the high-level dose group one female with an imperforate vagina and one male having a subcutaneous mass were killed in week 15 and 18, respectively.

In the F1 generation two control animals were killed in extremis. One male due to an accidental injury in week 2 and one female in week 15 due to difficulties with parturition (one dead foetus present in uterus).

Clinical observations

No treatment-related clinical signs of toxicity were noted.

During the pre-mating period, annular constrictions were visible on the tails of the F0 and F1 male and female rats. Almost all males and approximately half of the females, in all groups, were affected. Scaly tail was also observed in some of the animals. These findings were considered incidental to the administration of glyphosate acid in the diet. Other recorded changes in clinical condition were either isolated occurrences or of an incidence comparable with that of the control group.

These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.

Body weight

There was no effect of glyphosate acid on body weight adjusted for initial weight for the F0 rats, males and females, during the pre-mating period. For the F1 males given 10000 ppm, body weight was slightly lower at week 1, in comparison with the control group. Thereafter, body weights adjusted for initial weight remained lower than the controls for the duration of the pre-mating period and were statistically significant different from week 2 through to week 8 (see Table B.6.6-9). There was no effect of 10000 ppm on the body weight of the F1 females and no effect of 3000 or 1000 ppm on the body weight of the F1 males or the F1 females (see Table B.6.6-9). There was no effect of glyphosate acid on body weight adjusted for initial weight for either the F0 or F1 rats during gestation or lactation.

Table B.6.6-9: Body weight during the pre-mating period-F1 generation (Group mean values)

F1 generation	body weight (g)							
	Control		Low		Mid		High	
	(0 ppm)		(1,000 ppm)		(3,000ppm)		(10,000 ppm)	
Week	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)
1	80.2	74	81.1	75.2	78.1	74.2	75.3	73.4
2	130.1	115.4	132.3	115.7	128.6	114.7	127.6*	115.2
3	188.5	152.6	190.7	154.7	186.5	151.2	183.3*	152.3
4	246.2	178.3	247.6	180.2	242.8	176.5	237.3**	179.4
5	300.3	201	304.1	202.7	296.5	199.7	289.5**	202.1
6	345	219.8	347.5	224.1	334.5	217.2	328.7**	218.4
7	377.2	231.7	382.4	237.1	369	228.3	360.5**	234.4
8	403.6	241.9	410.1	245.1	395.3	237.2	387.0*	245.6
9	425	250.3	433.3	253.6	416.3	245.1	411.8	252.5
10	443.4	259.7	453.1	263.8	435.1	251.7	431.6	258.1
11	461.7	265.7	471.3	271.2	455.5	258.8	449.7	266.9

* - significantly different from control group $p < 0.05$

** - significantly different from control group $p < 0.01$

Food consumption

There was no effect of glyphosate acid on food consumption for the F0 generation, all F1 females and F1 males of the low and intermediate level dose group during the pre-mating period. Only F1 males of the high-level dose group showed significantly lower food consumption throughout the pre-mating period. There was no effect of glyphosate acid on food utilisation for the F0 generation, all F1 females and F1 males of the low and intermediate dose group during the pre-mating period. Food utilisation was slightly higher for F1 males given 10000 ppm glyphosate acid, the difference from control being statistically significant.

for weeks 5-8 only. There was no effect of glyphosate acid on food consumption for either the F0 or F1 rats during gestation or lactation.

Reproductive parameters

Oestrus cycle

There were no consistent toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation

There were no treatment-related effects on pre-coital interval, mating performance, and gestation length for both F0 and F1 generation animals.

Litter data

Size and Viability

No overt effects of glyphosate acid on pup survival or on litter size during lactation were detected.

In both generations the incidence of whole litter losses was low and similar across all groups. Glyphosate acid treatment did not affect the percentage of post-implantation loss. The proportion of F1A and F2A pups born live was slightly higher in the glyphosate acid group than in the control group. There was no effect of glyphosate acid on litter size at birth or during the time of lactation for either the F1A or F2A pups. The proportion of litters with all pups surviving and the proportion of pups surviving during lactation were also unaffected by the treatment. An increased proportion of litters with all pups surviving noted for the F1A litters in the 10000 ppm group in comparison with the control group were not present for the F2A litters since the F2A controls showed an improvement over the F1A controls. Sex distribution within the litters was not altered by the administration of glyphosate acid.

Growth and Development

There was no effect of glyphosate acid on pup weight at birth for the F1A or F2A pups. Thereafter, the bodyweights of the F1A pups in the 10000 ppm group were lower in comparison with the control group. The differences from control were statistically significant for males from day 8 through to day 29 and for females, from day 5 through to day 29. A similar effect was neither observed for the F2A pups in the 10000 ppm group nor for the F1A pups of the low and intermediate dose level groups. There was no effect of glyphosate acid on total litter weight of either generation. Also the day of age when preputial separation or vaginal opening occurred in the F1 parents was unaffected by treatment.

Clinical signs

No clinically observable signs of toxicity were noted for offspring from treated animals.

Pathology

Necropsy

No macroscopic findings that could be attributed to the treatment with glyphosate acid were observed in any animal of the F0 and F1 generation.

The incidence of unilateral pelvic dilatation was slightly higher (9/69) in F2A females in the 10000 ppm group compared with the other groups. Unilateral pelvic dilatation is a very common spontaneous change in the Alpk:APfSD strain of rat. There was no increase in incidence in the F0 or F1 adults or in the F1A pups and, as an isolated observation, it is considered incidental to treatment with glyphosate acid.

Organ weights

The treatment of rats with glyphosate acid did not affect the weight of the adrenal glands, brain, right cauda epididymis, epididymides, kidney, liver, ovary, pituitary gland, prostate gland, spleen, seminal vesicles, testes or uterus. For the F0 males given 10000 ppm glyphosate acid, liver and kidney weights adjusted for bodyweight were statistically significantly greater than in the control group. Similar changes were not observed in the F1 males given 10000 ppm glyphosate acid. Absolute and relative values were comparable with the control group (see Table B.6.6-10). The weight changes seen in the liver and kidney of the F0 males were therefore considered not to be treatment related. For the F0 males given 3000 or 10000 ppm glyphosate acid, brain weight adjusted for bodyweight was statistically significantly greater than in the control group. Absolute values were comparable with the control group (see Table B.6.6-10). Similar changes were not observed in the F1 animals. The weight changes seen in the brain of the F0 males were therefore considered to be incidental to treatment.

Table B.6.6-10: Liver, kidney and brain weights (relative and absolute) of males (Group mean values)

Dietary concentration (ppm)	No. of animals		Organ weight (g)					
			Liver		Kidney		Brain	
			Absolute	Relative	Absolute	Relative	Absolute	Relative
			F0 Generation					
0 (Control)	25	mean	19.3	3.4	3.20	0.57	2.11	0.38
		sd	2.6	0.2	0.38	0.04	0.09	0.03
1000	26	mean	19.1	3.5	3.17	0.58	2.12	0.39
		sd	2.3	0.2	0.36	0.04	0.08	0.03
3000	26	mean	18.7	3.5	3.11	0.58	2.12	0.40
		sd	1.9	0.2	0.27	0.03	0.07	0.03
10000	25	mean	19.7	3.6	3.23	0.59	2.13	0.40
		sd	2.7	0.2	0.38	0.04	0.07	0.05
			F1 Generation					
0 (Control)	25	mean	21.4	3.7	3.42	0.6	2.11	0.37
		sd	2	0.3	0.31	0.05	0.07	0.02
1000	26	mean	21.4	3.7	3.45	0.59	2.12	0.37
		sd	3.3	0.4	0.37	0.04	0.07	0.02
3000	26	mean	20.1	3.6	3.32	0.6	2.1	0.38
		sd	2.6	0.3	0.31	0.04	0.07	0.03
10000	26	mean	19.7*	3.6	3.36	0.62	2.1	0.39
		sd	2.3	0.3	0.28	0.04	0.07	0.03

sd - standard deviation

* - significantly different from control group $p < 0.05$

There was no effect of glyphosate acid on brain, spleen or thymus weight.

For the F1A female pups in the 10000 ppm group absolute thymus weight was statistically significantly lower than in the control group. There was no effect of glyphosate acid on the thymus weight of the F2A pups. The observation in the F1A females is therefore considered incidental to treatment with glyphosate acid.

Sperm assessment

In F0 and F1 males no effect of glyphosate acid on the number of sperm, sperm motility parameters or sperm morphology was observed.

Oocyte assessment

There was no effect of 10000 ppm glyphosate acid on the number of primordial and small growing follicles in the left ovary of the F1 parent animals.

Histopathology

No treatment-related changes were detected in the F0 and F1 generations.

Conclusion by the Notifiers

The oral administration of glyphosate acid to rats by dietary admixture at a maximum dose level of 10,000 ppm for two successive generations of the Alpk-APfSD rat resulted in possible treatment-related changes at 10,000 ppm, where a reduction in the bodyweight of the F1A pups in the 10000 ppm group with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the pre-mating period was observed. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 3000 ppm (equivalent to 322 and 459 mg/kg bw/day for males and females, respectively) for maternal and offspring for both the F0 and F1 generations.

Comment by RMS:

The study is considered acceptable and the evaluation is confirmed. Additionally, up to 10000 ppm over to successive generations no effect on the sexuality and fertility was observed in males and females.

Furthermore, the NOAEL for parental and offspring toxicity was considered to be 3000 ppm, which has to be converted into 293 mg/kg bw/d (mean daily intake of glyphosate during pre-mating phase in F0 males).

Comment by GTF on the first draft of the RAR (July 2013):

The GTF suggested that the appropriate NOAEL value should be based on 3000 ppm in F1 males, 352 mg/kg/day, and not on the achieved dose in the parental males of 293 mg/kg/day as proposed by the RMS.

RMS comment (August 2013):

The NOAEL 3000 ppm (293 mg/kg bw/d) was set for both offspring and parents (F1) on the basis of a reduction in body weight of F1 pups and a subsequent reduction in body weight of in F1 males. The dose of 352 mg/kg bw/d was only achieved in F1 males during pre-mating period (please refer to Table B.6.6-8).

Reference:

IIA, 5.6.1/03

Report:

██████████ (1997)

HR-001: A two-generation reproduction study in rats

████████████████████
Data owner: Arysta Life Sciences

Study No.: IET 96-0031

Date: 1997-06-19

not published

ASB2012-11495

Guidelines: OECD 416 (1981), US-EPA FIFREA Guidelines Subdivision F (1984), Japan MAFF Guideline 59 NohSan No. 4200 (1985)
Deviations: None
GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 1996-04-16 to 1997-03-31

Materials and methods

Test material:

Identification: Glyphosate technical, Code: HR-001

Description: White crystal

Lot/Batch #: T-950308

Purity: 94.61 % (w/w)

Stability of test compound: Not reported

Vehicle and/
or positive control: Plain diet

Test animals:

Species: Rat

Strain: Sprague-Dawley; Crj:CD (SD)

Source:

Age: 5 weeks

Sex: Males and females

Weight at dosing: Males: 132 - 148 g; females: 112 - 126 g

Acclimation period: 7 days

Diet/Food: Certified pulverized feed (MF Mash, Oriental Yeast Co., Ltd), *ad libitum*

Water: Filtered, sterilized well water, *ad libitum*

Housing:

During acclimatisation in groups of five per sex in suspended wire-mesh stainless steel cages). During pre-mating, and mating periods animals were housed in groups of 3/sex/cage. During mating one male and one female were housed in aluminium cages with wire-mesh floors and fronts. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were housed in pairs until termination. Males were housed up to four per cage after being used for mating.

Environmental conditions:

Temperature: 22 ± 2 °C

Humidity: 55 ± 10 %

Air changes: 15/hour

12 hours light/dark cycle

Study design and methods:

In life dates: 1996-04-16 - 1997-03-31

Animal assignment and treatment:

In a two-generation reproduction study groups of 24 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1200, 6000 and 30000 ppm HR-001 in diet. The dose levels were chosen based on results of a preliminary reproductive study in Crl:CD (SD) rats..

After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. The day of proved copulation was designated Day 0 of gestation. Copulated females were placed individually into breeding boxes with nestle material. The day of completed parturition was designated Day 0 of lactation. On Day 4 *post partum*, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on Day 21 of lactation and all F0 parental animals were sacrificed. Groups of 24 male and 24 female offspring from each dose group of the F0 generation were selected to form the F1 parents. Unselected offspring were sacrificed and subjected to a gross necropsy.

The offspring selected for the F1 generation were dosed for 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned on Day 21 of lactation and terminated together with F1 parental animals. F1 parental rats which failed to produce F2 offspring (10 males and 10 females with normal external genitalia and oestrus cycle) were mated with untreated rats of the same strain and sacrificed thereafter for fertility assessment (reproductive performance).

Diet preparation and analyses

Diets were prepared monthly during the pre-mating period, and biweekly during the breeding period. For each dose level a specified amount of the test substance was mixed with a small amount of basal diet in a mortar. This pre-mix was stirred into the remaining part of the diet. The diets were stored at about 4 °C in the dark. Analyses for homogeneity were done for each dose level of the first diet preparation. Analyses for achieved concentration were done for all prepared diets.

Clinical observations

A check for clinical signs of toxicity and mortality was made once daily on all F0 and F1 parental animals. A detailed physical examination was performed on males prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. Females were examined prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.

Body weight

Individual body weights F0 and F1 males adults were determined prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. F0 and F1 females were weighed prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.

Food consumption and compound intake

Food consumption for each cage was recorded and daily food consumption was calculated. Determination of food consumption was made on a weekly basis during the pre-mating period for males and females and during the breeding period for males. In addition, for females total

food consumption was determined at the following intervals: Day 0-7, 7-14, 14-20 of gestation and of days 0-7, 7-14 and 14-21 of lactation.

Compound intakes in parental animals were calculated during the pre-mating periods for each sex on a weekly basis.

Reproduction parameters

Oestrus cycle

The oestrus cycle was checked daily by microscopically examination of vaginal smears. Examinations were done for each female for one week prior to mating until copulation was confirmed.

Reproductive performance

Mating indices for males and females were calculated separately after copulation was confirmed. In addition, fertility and gestation indices, the length of gestation, as well as the number of implantation sites were determined.

Sperm assessment

An assessment of motility and morphology of epididymal sperm was done at necropsy for 10 males per group, which were selected for the organ weight measurement, as well as for males that failed to impregnate females.

Litter data

Total number of live and dead pups, and the number of males and females per litter were determined on Day 0 of lactation. The sex ratio was calculated for each group. Viability indices, were determined for each litter on lactation days 0, 4 and 21. Body weights were determined on lactation days 0, 4, 7, 14 and 21.

A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. A detailed physical examination was done on lactation days 0, 4, 7, 14 and 21.

Sacrifice and pathology

All surviving parental F0 and F1 males and females were sacrificed on Day 21 post partum and subjected to a gross pathological examination. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. The following organs and tissues were preserved: adrenals, aorta, brain, caecum, colon, duodenum, epididymis, eyes, gross lesions, head (incl. nasal cavity, paranasal sinuses, buccal mucosa and ears), heart, ileum, jejunum, kidneys, larynx, liver, lung, mammary gland, oesophagus, ovaries, pancreas, pharynx, pituitary, prostate, rectum, seminal vesicles, spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus (cornua and cervix) and vagina.

F1 and F2 pups that were not selected on Day 4 of lactation were also killed and necropsied on that day. In addition, F1 weanlings that were not selected for parental animals of the F1 generation and all F2 weanlings were necropsied at 22-26 and 21-26 days of their age, respectively. The same organs, as described above, were preserved from one animal per sex per litter of the F1 and F2 weanlings necropsied.

The following organs weights of 10 F0 and F1 males and females from each dose group that were sacrificed at the end of the study, as well as from pairs of parental animals that failed to

mate: adrenal gland, brain, epididymides, kidneys, liver, ovaries, prostate, pituitary, seminal vesicles (with coagulating gland and fluids), testes, uterus.

A histopathological examination was performed on the reproductive organs and pituitary of the control and high dose F0 and F1 parental animals that survived until scheduled termination. A histopathological examination of the reproductive organs and pituitary in the low and mid-dose group was only performed on pairs of animals that had failed to produce offspring.

In addition, a histopathological examination was performed on organs with significant weight change, and on all organs with gross pathological changes.

Statistics

One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Bartlett's test for equality of variance ($p=0.05$) followed by parametric analyses of variance in one-way classification ($p=0.05$) or Dunnett's t-test or Scheffé's multiple comparison test ($p=0.05$, 0.01 or 0.001); or Bartlett's test followed by Kruskal-Wallis test ($P=0.05$) and Dunnett-type mean rank test or Scheffé-type mean rank test ($p=0.05$, 0.01 or 0.001). Fisher's exact probability test ($p=0.05$, 0.01 or 0.001) and Mann-Whitney's U-test ($p=0.05$ or 0.01) were also used.

Results and discussion

Analysis of dose formulations

Based on the results of the dose-finding study the chemical stability of the test substance in the was given for 5 weeks (at room temperature) in sealed plastic bags in the dark, and for at least 2 weeks after being released from the plastic bags.

Homogeneity of the test substance in the dietary mixtures was satisfactory, percentage deviations from the overall mean were within 4%. The mean achieved concentrations of HR-001 in the diet preparations were in the range of 90 – 105 % of the nominal and therefore acceptable.

Mortality

F0 and F1 males

Seven unscheduled deaths occurred during the study. In the F0 generation one control male was killed for humane reasons during week 9 because it was found to have a ruptured eyeball. In the low level dose group one female was killed for humane reasons during week 14 having failed to litter on time, dead foetuses were present in the uterus. In the intermediate level dose group one female was killed in week 14 on gestation day 23 due to difficulties with parturition. In the high-level dose group one female with an imperforate vagina and one male having a subcutaneous mass were killed in week 15 and 18, respectively.

In the F1 generation two control animals were killed in extremis. One male due to an accidental injury in week 2 and one female in week 15 due to difficulties with parturition (one dead foetus present in uterus).

F0 and F1 females

There were no mortalities observed during the study period.

Clinical observations

F0 and F1 males

There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups.

At 30000 ppm F0 and F1 parental males exhibited loose stool with incidences during the pre-mating growth and breeding periods of 3/24 and 2/24 for the F0 generation, and of 13/24 and

0/24 for the F1 generation, respectively, with a significant difference in the value for the pre-mating growth period of the F1 generation. Since this finding was not observed in other groups including control, defecation of loose stool was considered to be treatment-related. Statistically significant differences were also observed in the incidence of hair loss during the breeding period for F0 males in all test substance groups. However, the occurrence of this change in the treated groups was rather lower than controls, and was considered to be incidental.

During the study period, one F0 male and one F1 male in the control group and one F1 male in the 6000 ppm group showed malocclusion of the incisors, respiratory wheezing, and red sebum. The aforementioned one F1 male of the 6000 ppm group also showed distension of the abdomen. These animals were euthanatised within several days after discovery due to unfavourable prognosis. Necropsy noted a fracture of the facial bones in all cases, suggesting that the alterations were caused by an accident in the cage. Accident malocclusion of incisors was also observed in one F1 male in the 1200 ppm group. However, test substance treatment of this animal was continued until termination of the study because its condition was improved.

F0 and F1 females

There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups. In F0 and F1 parental females, loose stool was also observed at 30000 ppm. The incidences during the pre-mating growth period and the lactation and post-weaning period were 1/24 and 6/24 for the F0 generation, and 4/24 and 2/24 for the F1 generation, respectively, with a significant difference in the value for the lactation and post-weaning period of the F0 generation.

Bodyweight

F0 and F1 males

Mean body weights of F0 and F1 males in the 30000 ppm group were consistently lower than those in the control group from treatment week 1 to the day of necropsy, and the differences from controls at treatment weeks 1-12 and 14 for the F0 generation, and treatment weeks 1-6 for the F1 generation were statistically significant. In the 1200 and 6000 ppm groups, mean body weights of F0 and F1 parental males were comparable to the controls throughout the study.

F0 and F1 females

There were no significant differences in mean body weights of F0 females in any treatment group when compared to control. In F1 females in the 30000 ppm group, mean body weight on lactation day 0 was significantly higher than that in the control group. In the 1200 and 6000 ppm groups, mean body weights of F1 parental females were comparable to the controls throughout the study.

Food consumption and test compound intake

F0 and F1 males

In F0 males, mean food consumption at treatment week 13 in the 1200 ppm group was significantly higher than that in the control group. Since there was no such increase observed in the mid- and high-dose groups throughout the study, this change was not thought to be treatment-related.

In F1 males in the 30000 ppm group, mean food consumption at treatment week 4 was significantly lower than that in the control group, but the values on the other treatment weeks

in this dose group were comparable to the controls. In the 1200 and 6000 ppm groups, mean food consumption of F1 males was comparable to the controls throughout the study.

F0 and F1 females

In F0 females, the values on treatment weeks 2-4 in the 30000 ppm group were significantly higher than the controls. Inversely, the value on lactation days 7-14 in this dose group was significantly lower than those in the control group. So it was unclear these changes were treatment-related or not. In the 1200 and 6000 ppm groups, mean food consumption of F0 females was comparable to the controls throughout the study.

In F1 females in the 1200 and 6000 groups, mean food consumption on lactation days 14-21 were significantly higher than those in the control group. However, these changes were thought to be incidental because no such increase was observed in the highest dose group. In the 30000 ppm group, mean food consumption of F1 females was comparable to the controls throughout the study.

The group mean achieved dosages are summarised in Table B.6.6-11 below.

Table B.6.6-11: Group mean achieved dose levels F0 and F1-generation

Group	Dietary concentration	Mean achieved dose level			
		(mg/kg bw/day)			
	(ppm)	Males		Females	
		F0	F1	F0	F1
Control	0	0	0	0	0
Low	1200	83.6	91.7	96.9	104.8
Intermediate	6000	417	458	485	530
High	30000	2150	2417	2532	2760

Reproductive parameters

F0 males and females

Reproductive performance of F0 parental animals was not adversely affected by test substance treatment, and no significant differences were observed in such parameters as percentage of females having normal oestrous cycle, mating index, fertility index, gestation index, duration of gestation, number of implantation sites, and number, motility and morphology of epididymal sperm between the control group and the treated groups.

F1 males and females

In F1 parental animals, reproductive parameters in the treated groups were also comparable to the controls with the exception of gestation index and number of implantation sites, on which some biases were occasionally observed.

The significant higher number of implantation sites at 1200 ppm when compared to control was considered to be unrelated to treatment, since there was no increase noted at 6000 and 30000 ppm.

A similar bias was also found in the fertility index. The fertility indices in the control, 1200, 6000 and 30000 ppm groups were 95.8 (23/24), 95.8 (23/24), 87.5 (21/24) and 79.2% (23/24), respectively, with somewhat low values in the 2 higher dose groups. However, these decreases were considered to be incidental because the differences between the control and treated groups were not statistically significant, and because, as described below, normal reproduction results were obtained in the F1 parental animals, which had failed to produce offspring in this study, after remating with untreated animals.

Among the total of ten F1 females mated with untreated males, only one female in the 30000 ppm group did not undergo pregnancy. Histopathological of this female showed no abnormalities in the reproductive organs and pituitary. So the cause of infertility of this female was not known. The other nine F1 females were proved to have normal reproductive performance. One F1 male in each of the 1200, 6000 and 30000 ppm groups could not successfully impregnate untreated females mated. These 3 males had histopathological abnormalities in the testes and epididymides, and abnormalities in the sperm parameters, as a cause of infertility. However, the other 7 males were proved to have normal reproductive performance. Thus, the majority of F1 males and females which had failed to produce offspring were proved to have normal reproductive performance.

Litter data

Number of pups delivered

Mean number of F1 and F2 pups delivered in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Sex ratio

Sex ratios of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Viability index

The viability indices of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Body weights

F1 pups

There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls. F1 pups of both sexes in the 30000 ppm group, showed significantly higher mean body weights on lactation day 0 than the controls. However, mean body weights on days 14 and 21 were significantly decreased when compared controls.

F2 pups

There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls during the lactation period. In F2 pups in the 30000 ppm group, mean body weights of both sexes on day 21 of lactation were significantly lower than those in the control group.

Clinical signs

There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group.

During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.

Pathology

Necropsy

F0 and F1 generation

Necropsy of parental animals of both sexes noted several findings in all groups including the control group. Among these alterations, the incidences of distension of the caecum in F0 and F1 males and females of the 30000 ppm group were significantly higher than those of the

controls, and were considered treatment-related. Statistically significant differences from controls were also found in the incidences of hair loss in F0 males of the 1200, 6000 and 30000 ppm groups. However, the values were rather lower than controls and were considered to be incidental. Other findings were low in their incidences and considered not treatment-related.

F1 and F2 pups

Necropsy of stillbirths found on lactation days 0, pups found dead during lactation days 1-4, and pups killed to reduce the litter size on lactation day 4 demonstrated no treatment-related abnormalities in any of the F1 and F2 pups.

During days 5-21 of lactation, only 2 F1 pups in the 1200 ppm group were found dead. Necropsy of these dead pups were not performed due to advanced autolysis.

Necropsy of F1 and F2 weanlings in the 30000 ppm group noted distension of the caecum, suggesting a treatment-related occurrence. In the 1200 and 6000 ppm groups, no treatment-related abnormalities were observed in any of the F1 and F2 weanlings.

Organ weights

F0 and F1 males:

There were no effects in the absolute and relative organ weights in F0 and F1 males of the low- and mid-dose groups. At 30000 ppm relative weights of the liver and kidneys of F0 and F1 males were significantly higher than the control values. These increases were considered treatment-related. In F1 males in the high-dose group, there was also a significant decrease noted in the absolute and relative weights of the prostate. Besides these changes, the relative brain weight of F0 males in the 30000 ppm group was significantly higher than the control value. However, this finding was considered to be the change associated with the low body weights in this group.

F0 and F1 females

In F0 females, the absolute and relative weights of all organs were comparable between the control and treated groups. In F1 females in the 30000 ppm group, the absolute and relative weights of the liver and kidneys were significantly higher than the controls, and these increases were considered treatment-related.

Significantly higher-than-control value was also observed in the absolute kidney weight in the 6000 ppm group. However, this increase was not considered treatment-related because statistical significance in the difference between the control and 6000 ppm groups disappeared when all F1 females were subjected to the weighing of the kidneys fixed in 10 % neutral buffered formalin. The significant lower relative ovarian weight observed in F1 females in the 1200 ppm group was considered to be an incidental finding because no such decrease was observed in the mid- and high-dose groups.

Histopathology

F0 and F1 generations

In all F0 and F1 males and females in the 30000 ppm group, histopathological examinations of the reproductive organs and pituitaries did not indicate any treatment-related alterations.

No treatment-related histopathological alterations were also evident in the following organs in which significant weight changes were detected: kidneys of F1 females in the 6000 ppm group; kidneys of F0 males and F1 males and females in the 30000 ppm group; and liver of F1 males and females in the 30000 ppm group.

Conclusion by the Notifiers

The oral administration of HR-001 to rats by dietary admixture at a maximum dose level of 30000 ppm for two successive generations of Sprague-Dawley rat resulted in maternal toxicity at 30000 ppm. Thus, the NOAEL for maternal toxicity is 6000 ppm, equivalent to 417 - 458 mg/kg bw/day and 485 - 530 mg/kg bw/day for males and females, respectively.

The NOAEL for reproduction is 30000 ppm, since the reproductive performance was not affected in any dose group. Based on the body weight effects and increased incidences of caecum distension the NOAEL for offspring is considered to be 6000 ppm.

Comment by RMS:

The study is considered acceptable. Parental toxicity was observed at highest dose of 30000 ppm (> 2000 mg/kg bw/d) only and consisted of loose stool (F0/F1, m/f), reduced body weight (F0/F1, m), caecum distension (F0/F1, m/f), increased liver and kidney weights (F0/F1, m/f), decreased prostate weight (F1). Histopathological alterations were not detected.

Reproductive toxicity was not observed up to the highest dose level, despite lower gestation indices of F1 females at mid dose of 6000 ppm (530 mg/kg bw/d) and high dose level. This finding was considered not to be treatment related, because most of the F1 animals which failed to produce offspring were proved to have normal reproductive performance after re-mating with untreated animals. Offspring toxicity was observed at highest dose level only and confined to reduced body weight and caecum distension in both sexes. Sexual maturation (preputial separation, vaginal opening) was not examined in this study. Based on the results the NOAEL for parental and offspring toxicity was considered to be 6000 ppm and for reproductive toxicity to be 30000 ppm (>2000 mg/kg bw/d).

Reference: IIA, 5.6.1/04

Report: [REDACTED] (1993)

Two Generation Reproduction Study in Wistar Rats.

Data owner: Feinchemie Schwebda GmbH

Study No: TOXI 885-RP-G2

Date: 1993-08-27

GLP: yes

not published

TOX9300009

Guidelines: OECD 416 (1983)

Deviations: None

GLP: yes

Acceptability: See RMS comment

Dates of experimental work: May 1991 - April 1992 (not further specified)

Materials and methods

Test material:

Identification:

Glyphosate technical

Chemical name

N-(Phosphonomethyl) glycine

Description:

Odourless, white crystal

Batch #:	60
Purity:	96.8 %
Date of receipt	11/9/1990
Stability of test compound:	More than two years at ambient temperature
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Rat
Strain:	Wistar rats (Random bred)
Source:	
Age at start of treatment (F0):	8 weeks
Sex:	Males and females
Mean body weight at initiation of dosing:	Males: 160 - 190 g; females: 141 - 160 g
Acclimation period:	7 days
Diet/Food:	Standard "Gold Mohur" brand powdered rat feed manufactured by M/s Lipton India Limited, Bangalore, India
Water:	Deep bore well water passed through activated charcoal filter and exposed to UV rays (Aquaguard on-line water filter cum-purifier manufactured by M/s Eureka Forbes Limited, Bombay, India) was provided in glass bottles <i>ad libitum</i>
Housing:	Groups of five/three rats of same sex per cage depending on the size of the animals were accommodated in standard polypropylene rat cages (size: L 430 x W 270x H 150 mm) with stainless steel top grill; bedding material (paddy husk) was changed three times per week.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40-70 % Air changes: 10-15/hour 12 hours light/dark cycle

Study design and methods:

In life dates: May 1991 to April 1992 (not further specified)

Animal assignment and treatment:

In a two-generation reproduction study groups of 30 Wistar rats per sex of the F0 generation received daily dietary doses of 0, 100, 1000 and 10000 ppm glyphosate technical in diet. After at least 8 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. The day of proved copulation (vaginal smear) was designated Day 0 of gestation. On Day 4 *post partum*, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on Day 21 of lactation and all F0 parental animals were sacrificed. Groups of 30 male and 30 female offspring from each dose group of the F0 generation were selected to form the F1 parents.

The offspring selected for the F1 generation were paired within each dose group to produce the F2 litters. F2 litters were weaned on Day 21 of lactation and terminated together with F1 parental animals.

Diet preparation and analyses

The required quantities of test compound were weighed and mixed manually with 10 kg of powdered rat feed to prepare the premix. The premixes were added to the bulk of remaining quantities of feed and mixed in ribbon mixer. Prepared feed bulks were sampled at different intervals for assaying test compound concentration in experimental diet.

Clinical observations

All animals were observed daily throughout the study and any visible clinical signs were recorded with details on type, severity, time of onset and duration. Any animal found dead or sacrificed *in extremis* was necropsied and macroscopically abnormal tissues were retained.

Body weight

Males were weighed weekly until termination. Females were weighed weekly during pre-mating, on Gestational Days 0, 6, 13, and 20 and on Days 1, 4, 7, 14 and 21 of lactation. Offspring were weighed sex-wise as litters on Days 1, 4, 7, 14 and 21 *post partum*.

Food consumption and compound intake

Food consumption for each cage of males was recorded weekly until termination. Food consumption of females was recorded weekly during pre-mating and at the following intervals: Days 0-6, 6-13, 13-20 of gestation and Days 1-4, 4-7, 7-14 and 14-21 of lactation.

Reproduction parameters

Reproductive performance

The following reproductive indices were recorded: Male and female fertility index, fecundity index, mean number of implantations, parturition percentage, percentage mortality of pregnant dams, percentage of live pups born, in females the pre-coital interval (time elapsed between initial pairing and detection of mating) and duration of gestation.

Litter data

Total number of live and dead pups, viability indices (mean viable litter size on day 0, live birth index), litter weight, individual sex and observations on individual pups (if any) were determined within 24 hours after birth. Survival indices were determined on Days 2, 4, 7, 14 and 21 of lactation. Body weights were determined on Lactation Days 0, 4, 7, 14 and 21. A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. On Day 4 *post partum*, offspring were culled to reduce litter size to eight.

Sacrifice and pathology

All surviving parental F0 and F1 males and females and the non-selected weanlings from F1 and all F2 weanlings were sacrificed and subjected to a gross pathological examination. Tissue collection was done for parent generation only. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible.

The following organs and tissues were preserved from all F0 and F1 parents of all groups: Ovaries, uterus, vagina, testes, epididymides, seminal vesicles, prostate, coagulation glands,

pituitary, adrenals, liver and kidneys. The organs were examined for gross pathological changes and those found abnormal were examined histopathologically.

Females failing to get mated within 21 days and females failing to produce a viable litter by Day 25 *post coitum* were necropsied and any macroscopically abnormal tissue was retained for histopathological examination. The presence of corpora lutea, implantations and resorptions was examined in females which had failed to produce a viable litter.

On Day 4 *post partum*, offsprings were culled to reduce litter size to eight, where possible; culled offspring or found dead were necropsied. All F2 pups were sacrificed at weaning.

Statistics

One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Dunnett's t-test (for body weight, food consumption, litter number, litter weight, gestation and lactation period), Z Test (for mating performance, fertility index, gestation index, live birth index, viability index, lactation index, pups survival data, number of dead pups at birth, survival indices, number littered) and χ^2 test (for dose-response relationship).

Results and discussion

Analysis of dose formulations

In-house stability study for glyphosate technical was carried out at 0, 2000 and 20000 ppm. Chemical stability was given for 30 days at room temperature with a loss of less than 7 % at 0, 2000 and 20000 ppm levels in experimental diet when stored in polyethylene lined stainless steel drums.

The mean achieved concentrations of glyphosate in the diet preparations were analysed; the achieved concentrations were in the range of 96-100 % of the nominal and therefore acceptable.

Mortality

F0 and F1 males

There were no deaths in male animals.

F0 females

In the females there were three deaths, two in the low dose group, (one dystokia and one suppurative pneumonia) and one in the high dose group (cause of death not ascertained).

F1 females

One dam in low dose group died of dystokia and no other mortalities were seen.

Clinical observations

F0 generation

Nasal discharge and snuffling and cannibalism were seen in all groups. No other treatment related changes in clinical signs were observed.

F1 generation

The incidence of clinical signs was low and not treatment or dose related.

Body weight

F0 males

Initial body weight of treatment groups was higher compared to the control group and this trend continued during the entire treatment period. The absolute weight gain (difference between initial and terminal) during entire treatment period was similar to control group in low and high dose while in mid dose it was slightly higher.

F0 females

No significant treatment related differences were noted between treated and control groups.

F1 males

Mid dose group body weight (both initial and subsequent weeks) was more than control. In high dose group initial body weight (Week 0) was higher than control but at Week 2 and 3 it was less. However in this group the body weight tended to be higher (not significant) during last seven weeks.

F1 females

The body weight of all treatment groups at selection (Week 0) was higher than in the control group and continued to be significantly higher than in the control group for up to Week 10 in mid and high dose groups. Body weights of the high dose group dams on Days 0, 6 and 13 of gestation period were significantly higher compared to controls but the body weight gain was statistically not significantly different. Another incidental significant finding was higher body weight (Gestational Day 0-20) of mid dose group dams compared to controls. Absolute body weight of mid dose group on Lactation Days 1 and 4 and that of high dose group during all periods of lactation was significantly higher than in control group. The mid dose group had lost body weight during Days 7-14, 14-21 and 1-21 of lactation period as compared to control.

Food consumption and test compound intake

F0 parents

Mean food consumption of males was comparable to the controls throughout the study. High dose female animals tended to consume significantly more food than controls during gestation. During lactation low and mid-dose females consumed significantly less than controls, especially for the Periods 7-14 and 14-21. High dose females consumed significantly more food for Lactation Days 4-7 as compared to controls.

F1 males

Treatment groups did not show consistent and dose related changes as compared to control group. However initially (Weeks 0-2) mid and high dose groups consumed significantly less feed and later on a few occasions mid dose group showed increased consumption.

Treatment group dams did not show treatment and dose related consistent difference from control group; on a few occasions the treatment groups showed both increased/decreased food consumption over control. During gestation there was no statistically significant inter group difference in feed consumption between control and treatments during gestation period. Low dose dams consumed significantly less food than controls during different lactation periods (except for Day 7 and Period 7-14). Mid and high dose group dams did not show any treatment and dose related changes over control except for an incidental finding of increased and decreased feed consumption on Day 7, 14 and Period 7-14 and 14-21 respectively in mid dose group.

Reproductive performance parameters of F0 parental animals such as female fertility index, number of implantations, gestation index, duration of gestation, live birth index, and duration of gestation were not significantly different between treated and control groups. Male fertility index was significantly higher in low and high dose groups over control.

On Day 1 of lactation, mean litter size was significantly less than control in low and mid dose groups and the mean viable litter size at birth was significantly less in low dose group; the number of live pups on Day 1 was significantly lower in the mid-dose group.

Reproductive performance parameters of F1 parental animals such as male and female fertility index, fecundity index, parturition percentage and mortality of pregnant dams was not different between treatment and control groups. The incidence of dams not littered tended to be higher in the mid-dose group compared to controls. A significantly decreased number of implantations was observed in low and mid dose groups; the percentage of live pups born was significantly reduced in the in mid dose group and significantly increased in the high dose group.

	Group 1 - control		Group 2		Group 3		Group 4	
	0 ppm		100 ppm		1000 ppm		10000 ppm	
	F0	F1	F0	F1	F0	F1	F0	F1
Number of dams in group	30	30	30	30	30	30	30	30
Number of dams littered	29	26	26	27	28	22	30	28
Mean litter size	11.3	11.7	9.8*	10.4	9.9*	10.9	10.4	11.9
Mean viable litter size at birth	11.0	11.7	9.7*	10.4	9.9	10.9	9.9	11.9
Number of pups alive on day 1	320	305	253	281	276*	239	296	334
Mean number of implantations	12.1	13.4	11.2	11.6*	11.0	12.0*	12.3	12.9
Percentage of live pups born [%]	87.9	87.6	83.5	86.5	86.5	79.7*	80.0*	92.8**

*significantly decreased; **significantly increased

*significantly decreased; **significantly increased

Litter data

Number of pups delivered

Mean number of F1 and F2 pups delivered and mean litter sizes in the 100, 1000 and 10000 ppm groups were comparable to the controls.

Sex ratio

Sex ratios of F1 and F2 pups in the 100, 1000 and 10000 ppm groups were comparable to the controls.

Viability index

F1 pups

In the low dose group the pup survival index for Days 4, 14 and 21 was significantly lower than in controls. In the mid dose group the live birth index and Day 14 survival index were higher and Day 4 survival index was lower compared to controls. In the high dose group on Day 14 and 21 survival index was higher than in controls. Dose response relationship was not seen in these parameters.

F2 pups

There were no statistically significant inter group differences between control and treatment groups in parameters of F2 litters at first observation including incidence of external abnormalities in pups. The mean number of pups (combined and individual sex) during different periods of lactation did not show statistically significant differences compared to control group.

The group mean values of pup survival data parameters like: live birth index, 24 hours survival index and survival index for Days 4, 7, 14 and 21 did not show any significant inter group difference between control and treatment groups.

Body weights

F1 pups

Mean litter weight of combined sex and female pups in treatment groups were significantly more than control group on Day 1 and 4, respectively. On Day 7 combined sex litter weight and male pup weight was significantly less than control in low dose group while in high dose group it was more than control group. On Day 21 the mean body weight of complete litter and individual sex pups of mid dose group were more than control group. None of these showed any apparent dose response relationship.

F2 pups

Combined sex litter weight on day one and that of female pups of all treatment groups was higher than in controls; in addition combined sex litter weight in low and mid dose groups and that of male and female in mid dose group was higher than control on Day 4. In high dose group the male pup body weight on Day 14 and 21 was lower than control. None of these parameters showed any dose response relations.

Clinical signs

There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group.

During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.

Pathology

Necropsy

F0 generation

The gross pathological lesions seen were consolidated lungs with ecchymoses, chronic liver changes, kidneys with cysts and dilated pelvis, and hypoplastic testes (1 in the control group, 2 in the mid-dose and 1 in the high-dose group). The incidence was low and did not appear to be compound or dose related.

F1 generation

The gross pathological lesions seen were consolidated and collapsed lungs with emphysema, hydronephrotic kidneys, and unilateral hypoplastic testes. The lesions observed were few and appeared to be incidentally. A single incidence of unilateral testicular hypoplasia was observed in each of the three treatment groups, hydronephrosis was seen in two animals in the high dose group.

F1 pups

A higher incidence of emaciated pups was recorded for the mid and high dose groups compared to controls. A low incidence of minor developmental abnormalities like Kinky tail, rudimentary tail, kidney hydro-nephrosis and dilated pelvis occurred without dose-response relation.

F2 pups

A higher incidence of emaciation has been observed in pups of high dose group. Occasional not treatment and dose related incidence of hydronephrosis and dilated pelvis in kidney have been recorded.

Histopathology

F0 generation

Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one control animal, two mid dose and one high dose animal. The control and high dose animals showed degenerative changes in the seminiferous tubules while the mid dose group were normal. These changes appeared to be incidental and not compound related.

F1 generation

Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one animal in each of the three treatment groups; the testes in the low and mid dose groups showed unilateral degenerative changes and giant cell formation in the seminiferous tubules and focal chronic inflammation. The testes in the high dose were normal though unequal in size. The changes appeared to be incidental and not compound related.

Conclusion by the Notifiers

The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations of Wistar-Dawley rats resulted in no maternal toxicity.

The NOAEL for reproduction is considered to be 10000 ppm, since the reproductive performance was not affected in a dose-related manner. The NOAEL for offspring is 10000 ppm, since no treatment-related effects on offspring could be observed.

Comment by RMS (Re-evaluation):

The previous evaluation in 2001 regarding the NOAELs is confirmed. However, this study is now considered supplementary, because an effect dose was lacking. The highest dose of 10000 ppm is considered to be the NOAEL for parental, reproductive and offspring toxicity. This dietary level would correspond to a mean daily compound intake of 700-800 mg/kg bw/d. [The mean daily intake was not reported for all dietary levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report].

Reference: IIA, 5.6.1/05
Report: [REDACTED] (1981)
 A three generation reproduction study in rats with glyphosate
 [REDACTED]
 Data owner: Monsanto
 Study No.: 77-2063; BDN 77-417
 Date: 1981-03-31
 not published
 TOX9552385
Guidelines: None (pre-guideline)
Deviations: Not applicable
GLP: no, pre-GLP
Acceptability: See RMS comment

Dates of experimental work: 1978-06-13 to 1980-04-09

Materials and methods

Test material:
 Identification: Glyphosate
 Description: Fine white powder
 Lot #: XHJ-64
 Purity: considered 100 % active ingredient for dosing preparations;
 Stability of test compound: Not reported
 Vehicle and/
 or positive control: Plain diet
 Test animals:
 Species: Rat
 Strain: CD® (Sprague-Dawley derived)
 Source: [REDACTED]
 Age at treatment initiation: 43 days
 Sex: Males and females
 Mean weight at initiation of dosing: Males: 139.9 - 144.3 g; females: 118.0 - 119.2 g
 Acclimation period: 7 days
 Diet/Food: Standard laboratory diet (Purina Lab Chow® 5001), *ad libitum*

Water: Automated watering system (Elizabethtown Water Company), *ad libitum*
Individually (except during mating and lactation), in elevated stainless steel wire mesh cages; nesting material.

Housing: Litter Kleen® hardwood shavings added to cages on Day 19 of gestation and changed when wet or soiled through Day 14 of lactation

Environmental conditions: 12 hours light/dark cycle
No details on temperature and humidity reported

Study design and methods:

In life dates: 1978-06-14 - 1980-04-09

Table B.6.6-13: Study design

Group	Dose level (mg/kg bw/day)	No. of adults initially assigned to mate F0, F1, F2		No. of matings per generation F0, F1, F2	Gross post-mortem examination	Histopathology of F0, F1 and F2 parents, F3b weanlings	
		Males	Females			Male	Female
1 Control (plain diet)	0	12	24	2	All	10	10
2	3	12	24	2		none	none
3	10	12	24	2		none	none
4	30	12	24	2		10	10

Animal assignment and treatment:

In a three generation reproduction study groups of 12 male and 24 female CD rats received beginning 63 days prior to mating of the F0 generation daily dietary doses of 0, 3, 10 and 30 mg glyphosate/kg bw in diet. Diet samples were taken at four week intervals for analysis of achieved test substance concentrations.

Mating: One male and two females of equivalent dose levels were caged together nightly until a sign of mating (sperm and/or copulation plug in the vagina) was observed or until 15 days had elapsed with no evidence of mating. The day on which evidence of mating was observed was defined as Day 0 of gestation.

In this study, the first litters (F1a, F2a and F3a) from each mating were raised to weaning and discarded. Rats produced by the second matings (F1b and F2b) were selected to become parents of succeeding generations or to be subjected to complete gross necropsy (F3b).

Diet preparation and analyses

Diets were prepared weekly during the study and were adjusted on the basis of body weight and food consumption.

Clinical observations

A check for clinical signs of toxicity and mortality was made twice daily. A detailed physical examination was performed on adult generations at weekly intervals throughout the study.

Body weight

Body weights of all animals were determined weekly during growth and rest periods of all generations. Pregnant females were weighed on Days 0, 6, 15 and 20 of gestation and lactating females were weighed on Days 0, 4, 14 and 21 of lactation.

Food consumption and compound intake

Food consumption was recorded weekly during growth and rest periods of all generations. Test substance intake was calculated from individual body weight and food consumption data and reported as a group mean value for weekly intervals during the growth and rest periods of all generations.

Reproduction parameters

The day on which evidence of mating was observed was designated as Day 0 of gestation; the day of delivery was designated as Day 0 of lactation.

Mating indices, pregnancy rates, length of gestation and male fertility indices were recorded.

Litter data

Pups of all generations were examined daily for general appearance and mortality. On Days 0, 4, 14, and 21 they were counted to record the number of live and dead pups. Body weights were determined on Days 0, 4, 14, and 21 as a litter and on Day 21 individually.

Total number of live and dead pups, and the number of males and females per litter were determined on Day 0 of lactation. The sex ratio was calculated for each group on Days 0 and 21 of lactation. Viability indices, were determined for each litter on Lactation Days 0, 4 and 21.

Sacrifice and pathology

Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. All adult males and females were sacrificed after pup selection of the last Fb litter, (F0, F1) and after last F3b litter weaned (F2) by lethal exposure to ether. Pups that were found dead or stillborn pups were weighed and given a gross post-mortem examination including internal sex determination, presence of milk in stomach. F1a, F2a, F3a and F3b animals were sacrificed at weaning, given a gross post-mortem examination and abnormal tissues were saved. F1b and F2b animals which were not selected as future parents were sacrificed after ensuing selection of parental animals, given a gross post-mortem examination and abnormal tissues were saved.

The following organs and tissues were preserved from all parents (F0, F1, F2) and from 10/sex/group of the F3b weanlings: adrenals, aorta, bone and bone marrow (sternal), brain, colon, duodenum, eyes with optic nerve and Harderian gland, gonads (ovaries and testes), heart, ileum, kidney (2), liver (2 sections), lung with main stem bronchi, lymph nodes (mesenteric), mammary gland (right inguinal), pancreas, pituitary, salivary gland, skeletal muscle (biceps femoris with right sciatic nerve), skin, spinal cord, spleen, stomach, thyroid/parathyroid, urinary bladder, uterus/prostate, gross lesions, tissue masses, thymus.

Microscopic examination of histological sections of these tissues were done for 10 male and 10 female animals from control and high-dose groups of F0, F1 and F2 parents and of F3b offsprings.

The following organs were weighed from all parents sacrificed after weaning of the second litters and from eighty F3b weanlings (10 males and 10 females per group): adrenals, gonads, kidneys, brain, spleen, liver, heart and pituitary.

All pups of the second litter of the F2 parents (F3b) were necropsied at weaning and specified tissues were preserved for selected animals in each group.

Statistics

Body weights, body weight gain, maternal body weights, food consumption, number of offspring, offspring body weights, terminal body weights and organ weight data (absolute and relative), offspring survival, litter survival, pup viability index at birth, mating indices, pregnancy rates and male fertility indices data were compared to the control. Statistically significant differences were evaluated using several methods including Dunnett's test, ANOVA, Barlett's test, Kruskal-Wallis test and Fisher Exact Test.

Results and discussion

Analysis of dose formulations

Not reported.

Mortality

F0 adults (2 dead females in mid-dose group)

In the F0 generation, no unscheduled mortality occurred in the control, low- or high-dose groups. One female of the mid-dose group died during on Lactation Day 20 of first litter having 13 live pups at time of death. A second female of the mid-dose group died on Lactation Day 7 of second litter; this female delivered eight pups - seven live and one dead - and all pups were dead at time of death. No mid-dose F0 male died.

F1 adults (1 dead female in mid-dose group, 1 dead female in high-dose group)

In the F1 generation, no unscheduled mortality occurred in the control or low-dose groups. In the mid dose group one female was killed in a moribund condition during the post-mating period for the second litter. This female had mated during the first mating but did not deliver a litter; during the second mating this female had not mated. No other mortality occurred in the mid-dose group. In the high dose group one female died due to an accident (animal was caught in the feeder jar). A second high-dose female died on Day 21 of gestation for the second litter; the uterus of this female contained 15 term foetuses. No other mortality occurred in the high-dose group.

F2 adults (1 dead female in low-dose group, 1 dead male in mid-dose group)

In the F2 generation, no unscheduled mortality occurred in the control or high-dose groups. In the low-dose group one female died during the F3a lactation period. This female delivered a litter containing only dead pups (13 pups) and died the day after parturition. No other mortality occurred in the low-dose group. In the mid-dose group one male was killed in a moribund condition during the period between mating of the first and second litters. This male had mated and impregnated both females during the first mating period. No other mortality occurred in the mid dose group.

Clinical observations

Clinical observation data were similar between the control and treated groups for each generation interval throughout the study. No adverse treatment effects were indicated.

Body weight

Mean body weight data during the growth and rest periods were comparable between the control and treated groups for each generation, throughout the study. Likewise, mean weight gain during the growth periods were comparable between these same groups for both sexes

throughout all generations. No treatment effect on body weight data during the growth and rest periods was evident.

Food consumption and test compound intake

Mean food consumption data were considered comparable between the control and treated groups (both sexes) during the growth and rest periods for each generation, throughout the study. No adverse effect of treatment on food consumption was evident throughout the study. Mean weekly test substance intake values ranged from 2.8 to 3.3 mg/kg bw/day for the low-dose group, from 9.5 to 11.2 mg/kg bw/day for the mid-dose group and from 27.7 to 33.1 mg/kg bw/day for the high dose group for all generations including both genders.

Reproductive parameters

Male and female mating indices and male fertility indices during both mating intervals of the F0 generation were considered comparable between the control and treated groups. During the second mating interval of the F0, pregnancy rates were lower than control in each of the treated groups; however, no indication of a dose-relationship was evident as the lowest pregnancy rate was seen in the mid-dose group. This reduction in pregnancy rate for the mid-dose group was not statistically significant. In the absence of a dose-response relationship the reduction in pregnancy rate during this mating interval (F1b) in the treated groups was not considered treatment-related.

In the F1 generation, mating indices (males and females) for both litter intervals were comparable between the control and treated groups. It is note-worthy that for both mating intervals of this generation, mating indices for control and some treated groups were lower than normally encountered in multi-generation studies. The reason for the poorer mating performance in this generation was unclear but no treatment effect was indicated since mating indices were lowest in the control group. Pregnancy and male fertility indices for the first mating interval of the F1 were comparable between the control and treated groups. During the second litter interval, pregnancy rates were lower than those seen for the first interval in control and treated groups. The lowest pregnancy rate was seen in the high-dose group; however, this difference from the control value was not statistically significant. Pregnancy rates for the low- and mid-dose groups, during the second mating interval, were considered comparable to control. Male fertility indices for this same mating interval were considered comparable between the control and treated groups.

In the F2 generation mating indices for the treated groups were lower than control for each mating interval. During the first mating interval of the F2 generation, the female mating indices were lower than control in each of the treated groups; however, only in the high-dose group was this difference from control statistically significant. The female mating index for the control group at this interval was 100 % which is higher than normally encountered. The female mating indices observed for the control group in this study have shown considerable variability ranging from 70.9 to 100 %. The poor mating performance for the treated groups during the first mating interval is attributed to two males in each treatment group that did not mate either female in their mating unit (each mating unit was comprised of one male and two females).

During the second mating interval of the F2 generation, male mating performance improved in the mid- and high-dose groups as both mid-dose males and one of two high-dose males that did not mate during the first mating interval, mated and impregnated at least one female. Male mating indices for the low-dose group remained unchanged as the same two males that did

not mate during the first interval, failed to mate during the second interval. Pregnancy and fertility indices for the treated groups were comparable to control for both litter intervals of the F2 generation.

Mean gestation length was comparable between the control and treated groups for each pregnancy interval in each generation. Over the entire study no consistent, dose-related effect was seen in mating, fertility or pregnancy indices to indicate an adverse effect of treatment.

Litter data

Litter size

Mean litter size data on Day 21 of lactation (weaning) was comparable between the control and treated groups for each litter interval throughout the study.

Sex ratio

Pup sex distributions ratios at Day 0 and 21 were generally comparable between the control and treated groups for each litter interval for each generation. No adverse treatment effect on sex distribution data was evident.

Viability index

The mean numbers of live, dead and total pups at birth and pup viability at birth for each pregnancy interval, were comparable between the control and treated groups for each generation. The litter survival indices were comparable between the control and treated groups for each lactation interval in the F0, F1 and F2 generation. In the F0 generation, postnatal survival indices for Days 0-4 and 4-21 were comparable between the control and treated groups for the first lactation interval (F1a). For the second litter interval of the F0, postnatal survival indices for the Day 0-4 interval were comparable between the control and treated groups. During the Day 4-21 interval, survival indices were significantly lower than control in each treatment group. The increase in pup mortality during this interval (i.e. Days 4-21) was attributed to high pup mortality within one or more litters at each treatment level. In the low-dose group the lower pup survival was attributed to one female that experienced complete litter mortality (litter contained 14 live pups at Day 4). In the mid-dose group, one female died on Day 7 of lactation and all seven pups in her litter died during the Day 4-7 lactation interval. Additionally three mid-dose litters lost five or more pups from their litters during the Day 4-21 lactation interval. In the high-dose group, one female lost nine of 12 pups during the Day 4-21 lactation interval.

In the F1 and F2 generations postnatal survival indices for Days 0-4 and 4-21 during both litter intervals were considered comparable between the control and treated groups. Some statistically significant differences in these indices were observed between the control and treated groups; however, no trend was evident through successive generations to indicate an adverse effect of treatment.

Body weights

Maternal body weights

Mean body weight data during the gestation and lactation intervals and mean weight change during these same periods were comparable between the control and treated group for each pregnancy interval from each generation throughout the study. No treatment effect was indicated in gestation - lactation body weight data throughout the study.

Offspring body weights

Mean pup body weight data during each litter interval for each generation were comparable between the control and treated groups. No adverse effects of treatment on pup weight data was evident.

Adult animals (F0, F1 and F2)

Mean terminal body weight data were comparable between the control and treated groups for both males and females throughout the study.

Pathology

Necropsy

F0, F1 and F2 generations

Gross necropsy of parental animals of both sexes did not indicate any adverse effect of treatment.

F1, F2 and F3 offspring

Gross post-mortem observations of offspring at weaning (F1a, F2a, F3a, F3b) or post-weaning (F1b, F2b) did not demonstrate an adverse effect of treatment. Likewise, evaluation of dead pups recovered at birth and during the 21-day lactation period did not note a treatment-related effect.

Organ weights

F0, F1 and F2 generations

Mean organ weight data (absolute and relative to body weights or brain weights) were comparable between the control and treated groups for both males and females from the F0 and F1 generations. Some statistically significant differences were noted between control and treated groups both in mean organ weight data and in the relative weight data; however, no trends were evident within dose levels or through these generations.

In the F2 generation, mean organ weight data (absolute and relative) for the males were comparable between the control and treated groups. In the F2 female group, mean liver/body weight ratios were significantly lower than control in each of the treated groups; however, no clear dose-relationship was apparent. Mean liver/brain weight ratios for the treated F2 females were lower than control; however, these differences from control values were not statistically significant. Mean spleen weights (absolute and relative to brain and body weights) were significantly higher than the control value in the F2 mid-dose female group; however, mean spleen weight data for the low- and high-dose F2 females were comparable to control values. In the absence of an effect on spleen weight in the high-dose Fg female group, the change seen in spleen weight data for the mid-dose females was considered spurious and not biologically meaningful. Other mean organ weight data (absolute and relative to body weight or brain weight) for the treated F2 female groups were considered comparable to control data.

F3b offspring

Mean organ weight data (absolute and relative to body weights or brain weights) were comparable between the control and treated groups for both males and females. No treatment-related effect was evident in organ weight data for the F3b offspring.

Histopathology

In total 160 male and female rats (40 adults of each generation F0, F1 and F2 and 40 weanlings of F3b) were examined microscopically. No microscopic findings were considered treatment related. Proliferative tissue changes diagnosed as neoplasms were few. The microscopic tissue alterations, neoplastic and non-neoplastic, were indicative of common incidental histological findings.

Conclusion by the Notifiers

The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 30 mg/kg bw/day for three successive generations of CD rats resulted in no treatment-related signs of toxicity in parental animals. The NOAEL for reproduction is 30 mg/kg bw/day, since the reproductive performance was not affected in any dose group. The NOAEL for offspring is 30 mg/kg bw/day, since no adverse effects on offspring were observed.

Comment by RMS (Re-evaluation):

This study is now considered not acceptable due to the selected dose levels that were much too low. Accordingly, an effect dose was not reached (Evaluation in 2001 not confirmed).

Reference:

IIA, 5.6.1/06

Report:

(1992)
The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat.

Data owner: Cheminova
Project no.: CHV 47/911129
Date: 1992-05-14
not published
TOX9552389

Guidelines:

OECD 416 (1983), US-EPA FIFRA 83-4 (1982)

Deviations:

None

GLP:

Yes

Acceptability:

See RMS comment

Dates of experimental work: 1990-03-29 - 1991-03-22

Materials and methods

Test material:

Identification:

Glyphosate technical

Description:

White solid

Lot/Batch #:

206-Jak-119-1

Purity:

99.2 %

Stability of test compound:

Stable during the treatment period.

Vehicle and/ or positive control:

Plain diet

Test animals:

Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) BR VAF/Plus
Source:	
Age:	Approximately 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 143 – 201 g; females: 106 – 175 g
Acclimation period:	At least 15 days
Diet/Food:	Biosure Laboratory Animal Diet No.2, <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	During pre-mating periods, animals were housed in groups of four in metal cages with wire mesh front, floor and top. During the first week of F1A and contingency animals of F2B animals were housed in plastic cages. During mating animals were housed on an 1:1 basis in plastic cages where females stayed after mating for breeding. Males were re-housed in former metal cages.
Environmental conditions:	Temperature: 23 ± 4 °C Humidity: 45 ± 24 % Air changes: not reported 12 hours light/dark cycle

Study design and methods:

In life dates: 1990-03-14 to 1991-03-22

Animal assignment and treatment:

In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate technical. The dose levels were chosen based on results of a previously conducted study. After at least 70 days of treatment pairing of animals within each dose group was undertaken on a 1:1 basis to produce the F1 litters. At Day 21 *post partum* of offspring from the F0 mating phase, groups of 24 male and 24 female offspring from each dose group were selected to form the F1A generation. The remaining pups were sacrificed. Approximately 10 days following the weaning of all F1A pups, F0 males and females were re-mated. At Day 21 *post partum* all F1B pups were sacrificed. F0 males and females were terminated shortly after weaning of F1B pups.

The selected F1A animals were dosed from approximately Week 4 of age for at least 84 days and then mated on a 1:1 basis (sibling pairings were avoided). On Day 4 *post partum* F2A litters were standardized to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 *post partum* all F2A pups were sacrificed. Approximately 10 days following the weaning of all F2A pups, F1 males and females were re-mated. On Day 4 *post partum* F2B litters were standardized to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 *post partum* all F2B pups were sacrificed. F1 males and females were terminated shortly after weaning of F2B pups.

Diet preparation and analyses

For the weekly preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 7 minutes in a rotary double-cone-blender.

The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration throughout the study.

Clinical observations

A check for clinical signs or ill health was made once daily and recorded daily for the first week of treatment and on a weekly basis thereafter. Rats showing marked signs of ill health or reaction to treatment were killed and subjected to necropsy.

Body weight

Individual body weights were recorded at the start of each generation (F0: Week 6 of age; F1A: Week 4 of age) and subsequent at weekly intervals. Females were weighed daily during mating and continued until parturition. Weights were reported for Days 0, 7, 14, 17 and 20 of pregnancy. Females with live litters were weighed on Days 0, 7, 14 and 21 *post partum*.

Food consumption and compound intake

Food consumption was recorded on a weekly basis from allocation throughout the first pre-mating phase of each generation. During this period food conversion ratios and achieved intake (mg/kg bw/day) were calculated.

Water consumption

Water intake was observed daily during the initial and final two weeks of the first pre-mating period for each generation and from allocation for the F0 generation.

Reproduction parameters

Vaginal smears were taken daily during the 20-day mating period to examine the oestrus cycle and median *pre-coital* time. Additionally, date of mating and duration of gestation was recorded.

Litter data

The number of offspring born and the number of offspring alive were recorded daily. Pups were weighed on Days 0 and 4 and all litters containing more than eight pups were culled to eight retaining, where possible, ideally 4 pups per sex. The remaining pups were also weighed on Days 8, 12, 16 and 21. Dead and culled young were subjected to necropsy.

Sacrifice and pathology

All adult animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded.

The following organs were weighed of adults: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate (with seminal vesicles and coagulating gland), testes (with epididymides), thymus.

The following tissues were preserved from all adults: adrenals, aorta, bone (femur and joint), bone marrow (sternum), brain, cranial vault (for lachrymal glands, teeth, nasal turbinates, inner ear), caecum, colon, duodenum, eyes, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (cervical/mesenteric), mammary gland, macroscopically abnormal tissues,

oesophagus, ovaries*, pancreas, pituitary*, prostate with seminal vesicles (with coagulating gland)*, rectum, salivary gland, sciatic nerve, skeletal muscle, skin, spinal column (vertebral column), spleen, stomach, testes (with epididymides)*, thymus, thyroids (with parathyroids), tongue, trachea (with larynx and pharynx), urinary bladder, uterus (with cervix)* and vagina*.

Histology of the reproductive tract was restricted to adults of the control and high-dose group and any apparently infertile animals at the lower dietary concentrations and confined to tissues marked with an asterisk (*).

Statistics

Two tailed significance tests were performed on adult parameters (water consumption, food consumption, bodyweight, organ weights) and litter data. Evaluation of other parameters were found not to be useful. Significances at 1 % and 5 % were reported.

Results and discussion

Analysis of dose formulations

Stability analyses indicated that the dose preparations at nominal concentrations of 500 and 30000 ppm were stable for up to 18 days during storage under animal room conditions.

Analyses for homogeneity at nominal concentrations of 500 and 30000 ppm indicated that the dose preparations were homogeneous.

Analyses for achieved concentration performed at 4-5 weekly intervals demonstrated that the prepared dietary admixture concentrations given to the animals were within ± 15 % of the nominal concentration in all groups.

Test compound intake

The group mean intakes of glyphosate are summarised in Table B.6.6-14 and Table B.6.6-15 below.

Table B.6.6-14: Group mean achieved intakes of glyphosate - F0 generation

Group	Dietary concentration (ppm)	Mean intakes (Week 1 - 10)	
		(mg/kg bw/day)	
		Males	Females
Control	0	0	0
Low	1000	66.4	75.3
Intermediate	3000	196.8	226.0
High	10000	668.1	752.3

Table B.6.6-15: Group mean achieved intakes of glyphosate - F1 generation

Group	Dietary concentration (ppm)	Mean intakes (Week 5 - 16)	
		(mg/kg bw/day)	
		Males	Females
Control	0	0	0
Low	1000	76.1	82.1
Intermediate	3000	230.2	244.9
High	10000	771.3	841.1

Mortality

There were no test substance related mortalities.

Four unscheduled deaths occurred during each generation.

In the F0 generation one female of the low-dose group and one male of the high dose group were killed for humane reasons during Week 15 and 23, respectively. The female exhibited pilo-erection and thin appearance and the necropsy noted thickened forestomach, invaginated stomach and abnormal contents in the gastro-intestinal tract. The male was unable to use hind limbs, exhibiting aberrations of brain and spinal cord at necropsy. Another male of the high-dose group died during Week 3, with effects on pancreas and liver noted at necropsy. One control male was sacrificed during Week 16 following poor condition, however, the aetiology of the signs was not established.

In the F1 generation one female of the low-dose group was killed following a procedural error. In the mid-dose group one male died during Week 34 but autolytic changes precluded a valid necropsy. Moreover, one male and one female died and were sacrificed, respectively, during Week 23. Necropsies failed to identify a specific cause of death.

Clinical observations

No treatment-related clinical signs of toxicity were noted. General signs were observed in occasional animals from both generations and were not related to treatment.

Body weight

No adverse effect of bodyweight change was evident for treated animals in comparison to controls for both generations.

However, absolute mean body weights in high-dose F1 males were slightly lower as compared to control. In addition it was noted that during the first mate of each generation, body weight gains during the initial stages of pregnancy tended to be slightly lower than controls at all dietary levels. Since no consistent dose-response was apparent these effects cannot conclusively be attributed to treatment.

Food and water consumption

Apart from a slightly higher but not statistically significant food consumption of high-dose F1 females during the second half of the pre-mating period, there were no marked intergroup differences in food consumption of males or females.

Apart from a slight increase among high-dose F1 females (attaining statistical significance in Week 16), no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations when compared to their concurrent controls.

Reproductive parameters

There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

Litter data

Size and Viability

No overt differences in litter viability were detected.

In the high-dose group total litter size at birth was consistently, but not significantly, lower than controls across all four matings and remained lower than controls at Day 4 in three of the four matings. Since the mean litter size at birth within each mating, was not always the lowest litter size recorded, this finding could not be clearly attributed to treatment.

Growth and Development

No adverse effects on mean offspring bodyweights, bodyweight change or development were detected for male and female offspring in comparison to their controls.

Clinical signs

No clinically observable signs of toxicity were observed for offspring from treated animals.

Pathology

Necropsy

There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1 animals, or offspring.

Organ weights

There were no overt or statistically significant treatment-related changes in any organ weights analysed in either generation.

Histopathology

No treatment-related changes in tissues associated with the reproductive tract were detected in the F0 or F1 generation animals.

Examination of two previously identified target organs, the parotid and submaxillary salivary glands, was initially performed only in the control and high-dose groups. Due to effects seen in the parotid gland, examination was extended to the remaining treatment groups. For the submaxillary gland, examination was extended to only the F0 and F1 females in the low- and mid dose group. The findings are summarised in Table B.6.6-16.

Table B.6.6-16: Incidence of salivary gland findings

Observation	Dietary concentration (ppm)							
	Males				Females			
	0	1000	3000	10000	0	1000	3000	10000
<i>F0 Generation</i>								
Animals examined	27	28	28	26	28	27	28	28
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid		2	3	12	0	2	5	17
submaxillary	0	-	-	0	0	1	4	14
<i>F1 Generation</i>								
Animals examined	24	24	23	23	24	23	24	23
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	1	0	4	11	0	0	4	9
submaxillary	0	-	-	0	0	0	0	3

- = not examined

Treatment-related minimal changes were apparent in the parotid salivary gland of both F0 and F1 males and females in the mid- and high-dose groups and the submaxillary salivary gland of the F0 females in the mid- and high-dose groups and F1 females in the high-dose group. This finding is similar to those seen occasionally in other subchronic and long-term dietary

studies and is considered to be an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and is not considered to be adverse. There were no effects on the salivary glands noted in the low-dose group.

Conclusion by the Notifiers

The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations resulted in minimal effects consisting of increased food and water consumption of F1 females, possibly reduced body weights of F1 males and minimal histological changes in the target organ (salivary glands) in F0 and F1 adults at 10000 ppm. The only findings associated with treatment at 3000 ppm were minimal histopathological changes of the salivary glands in F0 and F1 adults. No effects were apparent at 1000 ppm. Thus, the parental reproductive and offspring NOAELs are considered to be 10000 ppm, corresponding to 668 and 752 mg/kg bw/day in males and females, respectively.

Comment by RMS (Re-evaluation):

The study is considered acceptable. According to the study report, balano preputial cleavage was delayed in F1 males at all dose levels (42.5; 42.2; 42.6 d versus 39.9 d in controls). However, the calculated mean value for control animals seems to be reported incorrectly and should be calculated to be 41.8 d. Based on the revised mean value for preputial separation in control animals, no definite difference between the treatment groups was observed.

In conclusion, no evidence of reproductive effects was observed and therefore, the NOAEL on reproductive toxicity is considered 10000 ppm (668 mg/kg bw/d). The NOAEL for parental and offspring toxicity is calculated to be 3000 ppm (197 mg/kg bw/d) based on increased food and water consumption in F1 females, lower body weight of F1 males and an increased incidence of cellular alteration of the parotid (males and females) and submaxillary (females) salivary gland in both F0 and F1 adults at 10000 ppm (Evaluation in 2001 confirmed).

Reference:

IIA, 5.6.1/07

Report:

[REDACTED] (1990)

Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats

[REDACTED]

Data owner: Monsanto

Report No.: MSL-10387

Project No.: ML-88-106/EHL 88038

Date: 1990-08-27

not published

ASB2012-11496

Guidelines:

Not stated, but in general accordance with OECD 416 (1983)

Deviations:

Yes: no data on food efficiency; no details on fertility indices, number of live births and post-implantation loss, number of pups with grossly visible abnormalities,

GLP:

yes

Acceptability:

See RMS comment

Dates of experimental work: 1988-10-24 to 1989-10-13

Materials and methods

Test material:

Identification: Glyphosate (Identification code: T880068)

Description: White powder

Lot/Batch #: XLI-203

Purity: 97.67 %

Stability of test compound: Not reported

Vehicle and/
or positive control: Plain diet

Test animals:

Species: Albino Rat

Strain: Sprague-Dawley

Source:

Age: Approximately 7 weeks (F0 adults)

Sex: Males and females

Weight at study start (F0): Males: 165 – 207.6 g; Females: 135.6 – 162.7 g

Acclimation period: No data

Diet/Food: Purina Mills Certified RODENT CHOW No. 5002, *ad libitum*

Water: St. Louis public water, *ad libitum*

Housing: Housing for pre-mating and gestation (day 0 through 13): individual suspended stainless steel cages over paper bedding; during mating females were housed in the male's cages.
Housing for gestation and lactation (from day 14 of gestation through lactation): females housed in double wide cages with solid bottoms and wood shavings for bedding

Environmental conditions: Temperature: 18 - 26 °C
Humidity: 40 - 70 %
12 hours light/dark cycle

Study design and methods:

Animal assignment and treatment:

In a two-generation reproduction study groups of 30 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 2000, 10000 and 30000 ppm (corresponding to 132-140, 666-711, 1983-2230 mg/kg bw for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females (calculated from F0 and F1a adults)) glyphosate in the diet. After 11 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis in a male's cage for 7 days, to produce the F1a litters. If there was no evidence of mating after 7 days (copulatory plug, or vaginal smear), the female was co-housed with a male having recorded copulatory activity for additional 7 days, or until copulatory evidence was found. For F0 and F1 generation, gestation day 0 was set on the day on which copulatory evidence was found and lactation day 0 the day on which delivery of pups was completed.

At weaning of offspring from the F0 mating phase, groups of 30 males and 30 females offspring from each dose group were selected to form the F1 generation and the mating

procedure for F1a adults was conducted in the same way except modifications to exclude sibling matings. The remaining surviving F0 females and unselected offspring were terminated at Day 21 *post partum*. F0 males were killed at completion of mating phase. The offspring selected for the F1a generation were dosed for at approximately 14 weeks and then mated to produce the F2a and F2b litters (a second mating of the F1 generation was performed due to reduced litter sizes in pups from F0 of the 30000 ppm dose group). At weaning of the F2 litters all surviving adults and their offspring were killed, whereas F1 males were sacrificed after completion of mating phase.

Diet preparation and analyses

Approximately each week (except in one week when diets were prepared twice the same week and not during the following week) a known amount of glyphosate was mixed with the diet for 10 minutes in a HOBART HCM-450 mixing machine to achieve a batch size of 18 kilograms at each dose level.

The stability and homogeneity of the test substance in the diet were determined by liquid chromatography of duplicate samples from top, middle and bottom of mixer from the lowest and highest dietary levels stored in an open container at ambient temperature for 6 and 14 days or when frozen in a closed container for 35 days.

Clinical observations

A detailed observation for signs of toxicity was performed once weekly for the adult animals and for the offspring on days of weight measurement.

Body weight

Adult male animals of the F0 and F1a generation were individually weighted once weekly. The same was done for the female animals until copulation was confirmed, then females were weighted on days 0, 7, 14, and 21 of gestation and lactation.

Offspring was weighted on days 0, 4 (pre- and post-culling), 14 and 21 of lactation (except F1a males approximately two weeks prior to sacrifice and F1a females for approximately three weeks prior to mating for the F2b generation).

Food consumption and compound intake

Food consumption was recorded weekly for F0 and F1a adult males, except during mating, and also weekly for adult F0 and F1a female animals until mating. After confirmed copulation, the maternal food consumption was monitored for days 0-7, 7-14 and 14-21 of gestation and lactation, but it was not determined for females approximately three weeks prior to mating for the F2b generation and generally not for female animals that did not become pregnant.

Food conversion efficiency was not calculated.

Water consumption

No data on water consumption was given in the report.

Reproduction parameters

Pregnancy and parturition

Data on total paired females, females with confirmed copulation/total paired, pregnant/total paired, pregnant/ confirmed copulation was monitored as well as precoital (for pregnant animals) and gestational length in days. For males, the following items of interest were given:

males with confirmed copulation/total paired, males impregnating females/total paired and males impregnating females/confirmed copulation.

Litter data

The following litter data were recorded: Litter size, dead pups/litter, mean pup weight (on day 0, 4 (pre-/post-cull), 14, 21) and survival (%).

Physical and sexual development

No details on physical and sexual development of the offspring was reported.

Sacrifice and pathology

All adult animals, which died or were sacrificed in moribund condition were subjected to a gross necropsy and selected tissues were sampled. Pups found dead or culled pups also underwent gross pathology, but no tissues were saved. No organ weights were determined.

All F1a weanlings, that were not selected for mating, F2a and F2b weanling pups as well as females which had littered on or after 21 of lactation were sacrificed as scheduled. Non-pregnant adult females were killed at least 5 days after last expected parturition date and adult males after completion of the mating phase.

External and internal cavities of the dead animals were opened and the organs were examined in place and then removed. Hollow organs were opened and examined. The following organs of F0 and F1a males and females from each dose group that were sacrificed at the end of the study sampled, were weighed: ovaries and testes with epididymides. When present, the following organs from the F0 and F1a adults (unscheduled deaths and scheduled sacrifice) were retained: kidneys, ovaries, prostate, seminal vesicle, skin/mammary gland, testes, epididymis, uterus/vagina and gross lesions (pituitary retained for F1a adults only). Tissues from the F1a weanlings were saved at the discretion of the necropsist. From the F2a and F2b weanlings, which were sacrificed at schedule, the kidneys of 1 pup per sex and litter were saved.

A histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on one F2b weanling/sex/litter (selected at random) as well as on all retained tissues from unscheduled adult deaths. For preparation, fixed tissues were washed, dehydrated, embedded in paraffin, sectioned, stained with haematoxylin and eosin and examined under light microscopy.

Statistics

Dunnett's multiple comparison test (two-tailed) was used to detect statistically significant differences in adult body weights and food consumption between treated animals and their respective control.

Terminal body weights, maternal body weights and food consumption during gestation and lactation, pup weights, precoital length, gestational length, litter size, dead pups/litter, pup survival, absolute organ weights and organ/body weight ratios were evaluated by decision-tree statistical analyses procedures which, depending on the results of tests for normality and homogeneity of variance [Bartlett's Test], were chosen either parametric [Dunnett's Test and Linear Regression] or nonparametric [Kruskal-Wallis, Donckheere's and/or Mann-Whitney Tests] routines to detect differences and analyzed for trend.

The uncorrected Chi-Square test was used to examine fertility indices, e.g. females/males with confirmed copulation/total paired, pregnant/confirmed copulation (females) and males impregnating females/total paired as well as males impregnating females/confirmed copulation.

Fisher's Exact test with Bonferroni Inequality Procedure was used for statistical analysis of microscopic lesions.

Other statistical routines used for some data included: Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample (group) means could be considered as an estimate of a common population, and Grubb's Test to detect outliers.

Results and discussion

Analysis of dose formulations

The analysis of the test substance stability conducted over the time span of the study indicated that the test material was stable in the diet and homogeneity was adequate for study use. The stability of the test material in the diet was demonstrated at the low and high dose level, stored in an open container at ambient temperature for 6 and 14 days, or when frozen in a closed container for 35 days.

Analysis for achieved concentrations, demonstrated that the test substance-levels in the prepared diet were in the range of 95 to 96.7 % of the nominal concentration.

Test compound intake

The group mean achieved dosages are summarised in the table below.

Table B.6.6-17: Group mean compound intake levels during pre-mating periods of F0 and F1

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*			
		F0		F1	
		Males	Females	Males	Females
control	0	0	0	0	0
low	2000	132	160	140	163
mid	10000	666	777	711	804
high	30000	1983	2322	2320	2536

* based on actual food intake and body weight data; values were calculated in the report

Mortality

There were no treatment-related mortalities.

One female of the F0 generation died early in the study. This animal was never mated and at necropsy changes in bladder in kidneys were observed. Two male animals of the 2000 and 30000 ppm dose groups (F1 generation) died. Necropsy of these animals noted thymus and respiratory changes. One female animal of the F1 generation (2000 ppm) was sacrificed in extremis and another female (same generation, same dose group) died. Kidney changes and retained foetus; pups in uterus and stomach changes, respectively, were observed in these two females.

Concerning the offspring, dead pup counts at day 0 and survival of all F1a, F2a and F2b treated pups were not adversely affected when compared to the controls.

Clinical observations

The only clinical signs that were related to the test substance were soft stool in the animals of the 30000 ppm dose group. Other clinical signs, such as red ocular discharge / laboured respiration / overgrown teeth / piloerection / abrasions / emaciated and dehydrated appearance / misuse of limbs / focal loss of hair / swollen feed, occurred sporadically and were not considered to be treatment-related.

Body weight

At the highest exposure level of 30000 ppm, reduced body weights were observed in both sexes and in F0 and F1 generation. In the F0 generation, body weights gradually decreased within time to approximately 8 % less than controls prior to mating. F0/F1 weaning animals were lighter in weight as their corresponding controls and maintained that weight difference (approx. 10 % less than control) until the end of the study (see Table B.6.6-18).

No test-substance related body weight effects were observed in the adult animals of the 2000 and 10000 ppm dose groups prior to mating.

During gestation and lactation, maternal body weights in the highest dose group tended to remain lower than in controls, but the animals showed a rather greater body weight gain than the controls during gestation and lactation so that by the end of lactation, body weights were approximately the same as those of the controls (see Table B.6.6-19 and Table B.6.6-20).

Terminal body weights were significantly decreased for both sexes at the highest exposure level (see Table B.6.6-18).

Table B.6.6-18: Mean group body weights

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day					
			0	72	T [#]	0	72	T [#]
			F0 Generation					
			Males			Females		
0 (Control)	30	mean	187.9	494.6	549.56	150.5	276.7	296.31
		sd	11.65	34.86	46.76	6.86	23.85	23.63
2000	30	mean	188.1	497.6	550.19	150.5	272.6	290.64
		sd	11.35	49.87	80.72	7.03	22.86	19.50
10000	30	mean	188.1	484.4	539	150.2	273	290.71
		sd	11.57	42.13	58.13	7.04	27.92	25.35
30000	30	mean	188	455.8**	503.51**	150.3	253.8**	265.91
		sd	11.66	46.46	45.66	7.06	18.46	15.44
			F1 Generation					
			129	219	T [#]	128	219	T [#]
0 (Control)	30	mean	118.3	534.7	625.04	99.8	285.8	316.21
		sd	26.11	38.84	53.11	17.44	27.63	37.37
2000	30	mean	115.2	540.3	632.14	96.7	282.1	313.74
		sd	16.2	44.9	74.57	11.47	24.5	30.53
10000	30	mean	114.8	514.1	590.98	97.1	275.9	312.36
		sd	17.42	58.31	70.06	14.18	20.55	26.71
30000	30	mean	104.9*	483.4**	543.40**	88.8*	253.7**	284.72**
		sd	19.79	41.32	58.12	16.32	19.56	18.04

*: Dunnett's test (two-tailed) indicates statistically significant difference (p<0.05)

**: Dunnett's test (two-tailed) indicates statistically significant difference (p<0.01)

[#]T: Termination

Table B.6.6-19: Mean maternal body weights during gestation

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day (Gestation)			
			0	7	14	21
			F0 Generation			
0 (Control)	24	mean	274	301.83	324.41	398.26
		Sd	24.26	24.58	22.85	26.12
2000	29	mean	272.72	297.33	319.90	392.86
		Sd	20.52	21.71	19.84	24.28
10000	28	mean	271.80	299.22	323.43	395.08
		Sd	24.12	26.40	28.44	25.87
30000	28	mean	255.05**	282.44**	305.83**	375**
		Sd	16.49	16.27	17.44	24.70
			F1 Generation (First Mating)			
			0	7	14	21
0 (Control)	24	mean	285.29	308.95	328.70	392.56
		Sd	25.48	26.58	29.18	36.19
2000	29	mean	278.65	304.40	324.15	383.45
		Sd	23.42	23.48	25.06	28.18
10000	28	mean	268.89*	297.23	319.08	382.71
		Sd	19.24	18.81	19.27	21.77
30000	28	mean	251.30**	276.28**	299.48**	360.46**
		Sd	17.42	18.92	19.29	33.31
			F1 Generation (Second Mating)			
			0	7	14	21
0 (Control)	24	mean	324.22	340.99	363.44	428.99
		Sd	23.11	27.81	27.98	36.87
2000	29	mean	315.21	338.27	360.35	426.88
		Sd	26.06	28.67	28.39	33.67
10000	28	mean	305.27*	333.66	357.50	428.51
		Sd	20.26	22.45	24.49	26.17
30000	28	mean	281.46**	308.92**	330.95**	393.67**
		Sd	17.79	22.19	22.36	34.88

Table B.6.6-20: Mean maternal body weights during lactation

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day (Lactation)			
			0	7	14	21
			F0 Generation			
0 (Control)	24	mean	299.96	319.59	317.33	313.39
		Sd	23.21	23.58	28.96	20.01
2000	29	mean	297.48	317.91	314.53	313.96
		Sd	21.10	18.66	25.22	16.63
10000	28	mean	298.78	315.15	312.41	319.10
		Sd	20.81	22.04	22.94	18.61
30000	28	mean	285.84*	307.64	304.75	316.68
		Sd	13.91	12.48	20.68	15.43
			F1 Generation (First Mating)			
			0	7	14	21
0 (Control)	24	mean	299.29	313.60	337.68	313.49
		Sd	27.02	26.12	25.31	21.38
2000	29	mean	295.16	308.28	332.10	314.69
		Sd	23.58	22.56	23.92	23.95
10000	28	mean	296.63	310.80	328.29	313.14
		Sd	19.01	18.64	18.33	14.06
30000	28	Mean	277.91**	289.88**	315.88**	306.15
		Sd	17.89	17.23	17.47	20.18
			F1 Generation (Second Mating)			
			0	7	14	21
0 (Control)	24	mean	342.78	343.21	353.34	337.16
		Sd	32.46	27.11	21.15	17.22
2000	29	mean	340.16	336.62	348.40	331.96
		Sd	28.54	16.11	25.89	20.67
10000	28	mean	333.80	342.41	352.70	334.56
		Sd	23.35	26.93	20.43	13.82
30000	28	mean	312.39**	324.09*	337.08	329.95
		Sd	23.73	20.50	19.09	18.41

Food consumption

Overall, food intake was not notably affected during the study.

All animals of the 30000 ppm dose group consumed about 1 to 2 grams/day less than controls. This effect was mostly pronounced in the first week of exposure and also observed in the F0 dams. Subsequent dams (F1 first and second matings) tended to eat similar or larger amounts of the diet than controls.

No effects on food consumption were observed in the animals of the 2000 and 10000 ppm dose groups.

Reproductive parameters**Mating Performance, Fertility, Gestation and Lactation**

No effects on mating and fertility rates were observed in the F0 and F1a dams when compared to controls and no effects were observed on precoital length at any treatment level.

Litter data**Size and Viability**

Day 0 dead pup counts among treated groups were comparable to the control group for all three litters of pups (F1a, F2a and F2b generation).

A slight reduction in the average litter size was observed in the F0 dams of the 30000 ppm dose group. This effect was less pronounced in animals after the first F1 mating. Although the

difference was not statistically significant and not accompanied by an increase in dead pups/litter, a treatment-related effect could not be excluded. Therefore a second mating of the F1a adults was performed. In the resulting F2b generation, no dose-related decrease in litter size was observed.

Growth and Development

Birth weights and initial growth rate for pups from the treated dams compared well to the ones of the control, except the pups of the 30000 ppm dose group had reduced body weights on day 21 of lactation (more than 10 % difference to controls). The effect was earlier pronounced in the F1 matings (day 14). This effect was reasoned by the titrated uptake of the test substance-containing diet at the end of lactation.

In the mid dose group, slight and transient decreases in the body weights of the pups were observed. They were not evident in both sexes from all generations and therefore regarded of questionable toxicological significance.

Table B.6.6-21: Mean pup weights

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day			
			0		21	
			Males		Females	
			F0 Generation			
0 (Control)	24	mean	6.28	53.39	6.96	50.80
		Sd	0.49	3.90	0.52	4.39
2000	29	mean	6.27	51.82	6.91	49.47
		Sd	0.48	5.26	0.48	5.05
10000	28	mean	6.43	50.42*	6.15	49.16
		Sd	0.47	3.66	0.50	3.12
30000	27	mean	6.47	46.30**	6.12	44.99**
		Sd	0.62	4.09	0.59	4.34
			F1 Generation (First Mating)			
0 (Control)	28	mean	6.33	55.11	5.95	51.93
		Sd	0.60	5.64	0.55	5.07
2000	23	mean	6.20	52.47	5.90	51.42
		Sd	0.76	9.15	0.70	4.08
10000	22	mean	6.32	51.53*	5.98	48.49*
		Sd	0.74	7.35	0.64	5.93
30000	25	mean	6.50	47.29**	6.05	44.41**
		Sd	0.84	4.62	0.74	4.90
			F1 Generation (Second Mating)			
0 (Control)	16	mean	6.48	55.03	6.04	49.35
		Sd	0.75	6.38	0.63	10.96
2000	18	mean	6.17	52.74	5.86	50.73
		Sd	0.74	6.12	0.83	5.91
10000	17	mean	6.36	52.29	5.92	49.48
		Sd	0.52	3.35	0.47	2.52
30000	23	mean	6.51	44.43**	6.04	43.10**
		Sd	0.63	6.86	0.55	3.81

Clinical signs

No clinical signs were observed in the offspring of treated animals.

Pathology

Necropsy

There were no toxicologically significant macroscopic gross lesions attributed to the test chemical administration.

Organ weights

There were no statistically significant organ weight changes, except a slight increase in testes to body weight ratios in F1a adults of the 30,000 ppm dose group. This effect was attributed to their lower terminal body weight.

Histopathology

No treatment-related changes were detected.

Conclusion by the Notifiers

The oral administration of glyphosate to rats via diet at a dose levels of 2000, 10000 and 30000 ppm for two successive generations resulted in possible treatment-related changes at the maximum dose of 30000 ppm. A high incidence of soft stools in adults was accompanied by consistent reduction of body weights of adults and pups at this dose level. Decreases in body weights of the pups obviously occurred at the end of lactation, with the beginning of consuming the test substance-containing diet. Furthermore, slightly but not statistically significant reduced average litter size was noted in F0 dams of the 30000 ppm dose group at first mating.

Therefore the NOAEL was considered to be 10000 ppm for adult toxicity for both the F0 and F1 generations (corresponding to 666-711 mg/kg bw for males and 777-804 mg/kg bw/day for females).

The NOAEL for reproductive toxicity, for both generations and offspring was considered to be 30000 ppm.

The NOAEL for developmental toxicity, for both generations and offspring was considered to be 10000 ppm.

Comment by RMS (Re-evaluation)

The study is considered acceptable. In contrast to the Notifiers, the mid dose of 10000 ppm is considered the NOAEL for parental, offspring and reproductive toxicity. This assessment is in accordance with the evaluation in the previous DAR (2001, ASB2010-10302) and based on reduced body weight gain and soft stools in high dose adults, decreased pup body weight gain and equivocally reduced litter size at highest dose level of 30000 ppm. The intermediate dietary concentration of 10000 ppm was calculated to be 722 for male rats and 757 mg/kg bw/d for females.

B.6.6.2 Separate male and female studies

Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

B.6.6.3 Three segment designs

Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

B.6.6.4 Dominant lethal assay for male fertility

Studies considered not necessary. Information provided in chapter IIA 5.4.6.

B.6.6.5 Cross-matings of treated males with untreated females and vice versa

Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC

B.6.6.6 Effects on spermatogenesis

Studies considered not necessary. Effects on spermatogenesis are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

B.6.6.7 Effects on oogenesis

Studies considered not necessary. Effects on oogenesis are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

B.6.6.8 Sperm motility and morphology

Studies considered not necessary. Parameters are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

B.6.6.9 Investigation of hormonal activity

Separate studies considered not necessary. The potential hormonal activity is assessed in two-generation and developmental toxicity studies (see IIA 5.6.1, IIA 5.6.10 and IIA 5.6.11).

B.6.6.10 Teratogenicity test by the oral route in the rat

For the previous EU evaluation, a total of 5 studies in rats were reported in the DAR of which four were still considered acceptable or at least supplementary and may be used for current evaluation. The previous assessment of the studies by [REDACTED] (1991, TOX9552393) and by [REDACTED] (1980, TOX9552392) and of the [REDACTED] study (1981, TOX9650160, Author's name perhaps [REDACTED] but not verified) were confirmed by the RMS upon re-evaluation. However, during re-evaluation the study by [REDACTED] (TOX9551834) was considered not acceptable due to several reporting deficiencies and therefore not reported in the present RAR.

In addition, two new studies in rats have been performed and were provided in the GTF dossier. These studies were submitted either for the first time for this evaluation or had been subject to JMPR evaluation in 2004 yet. They were considered acceptable by the RMS without restrictions.

Reference: IIA, 5.6.10/01
Report: [REDACTED] (2002)
 Amendment 001 to Glyphosate Acid: Developmental Toxicity Study in the Rat
 [REDACTED]
 Data owner: Syngenta
 Report No.: CTL/P/4819
 Date: 2002-11-20
 GLP:
 not published
 ASB2012-10080
Guidelines: OECD 414 (2001); OPPTS 870.3700 (1998); 2004/73/EC B.31 (2004)
Deviations: None
GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 1995-05-17 to 1996-03-26

Materials and methods

Test Material: Glyphosate acid
 Description: Technical, white solid
 Lot/Batch number: P24
 Purity: 95.6 % w/w a.i.
 CAS#: Not reported
 Stability of test compound: Confirmed
 Vehicle and/or positive control: Deionised water.
 Test Animals:
 Species: Rat
 Strain: Alpk:APfSD Wistar-derived
 Age/weight on arrival: Approximately 11 weeks / 210 – 303 g
 Source: [REDACTED]
 Housing: Individually
 Acclimatisation period: Not applicable
 Diet: CT1 diet (Special Diet Services, Witham, Essex, UK) *ad libitum*
 Water: Mains water *ad libitum*
 Environmental conditions: Temperature: 21 ± 2 °C
 Humidity: 40 - 70 %
 Air changes: 25 – 30 changes / hour
 Photoperiod: 12 hours light / 12 hours dark

Study design and methods

In-life dates: Start: Not reported End: Not reported (QA audits conducted between May 1995 and March 1996)

Mating procedure: Virgin female rats were paired overnight (at the Breeding Unit) with males of the same strain. On the following morning, vaginal smears from these females were examined for the presence of sperm. The day when spermatozoa were detected was designated day 1 of gestation and, on this same day, successfully mated females were delivered to the experimental unit at CTL.

Animal assignment: A total of 96 mated females was supplied over a two week period. Twelve female rats were supplied on each of eight days. The study was divided into twenty four replicates (randomised blocks) with each replicate containing one rat from each group. Animals were randomly assigned to test groups as shown in the following table.

Table B.6.6-22: Animal numbers and treatment groups

Dose level of Glyphosate acid (mg/kg bw/day)			
0 (control)	250	500	1000
1 - 24	25 - 48	49 - 72	73 - 96

Dose selection rationale: The dose levels selected for this study were based on a dose range finding study in the pregnant rat. The highest dose level of 1000 mg/kg/day is the limit dose for this type of study.

Dose preparation and analysis: Glyphosate acid was administered in deionised water and the concentration was adjusted to give a constant volume of 1 mL/100 g bodyweight for each dose level. An appropriate amount of deionised water was added to a weighed amount of test substance (adjusted for purity) to provide each preparation. One preparation per concentration (ie 25, 50 and 100 mg/mL) was made. Each preparation was thoroughly mixed before being subdivided into aliquots. The control substance was also dispensed into aliquots. The aliquots were stored at room temperature and fresh aliquots were used for each day of the study.

A sample of each preparation was analysed prior to the start of dosing to verify the achieved concentrations of glyphosate acid in deionised water. Samples of the lowest concentration of dosing formulation was analysed to confirm the homogeneity of glyphosate acid in deionised water. The homogeneity of the 100 mg/mL formulation was not determined as part of this study and the data have been obtained from a preliminary study (*Moxon ME, 1995, Study cited but not submitted*) for which the method of preparation of the dosing formulations was the same. The chemical stability of glyphosate acid in deionised water was determined by re-analysis of the lowest and highest concentrations of dosing formulation after an interval of 26 days.

Concentration analysis results: The achieved concentrations of glyphosate acid in deionised water were within 5 % of nominal concentrations.

Homogeneity results: The homogeneity of glyphosate acid in deionised water at concentrations of 25 mg/mL and 100 mg/mL was within 5 % of the overall mean.

Stability results: The stability of the 25 mg/mL and the 100 mg/mL formulations was satisfactory over a period of 26 days which exceeded the period of use in this study.

Dosage administration: All animals were dosed once daily from days 7 – 16 (inclusive) of gestation with 1 mL of dosing formulation per 100 g bodyweight using a disposable syringe and a plastic nelaton catheter. The volume given to each animal was adjusted daily according to body weight. Control animals received the appropriate volume of deionised water. Dosing

was performed in group order with all animals receiving the same dose level being dosed sequentially.

Observations:

Maternal observations: All animals were observed on arrival to ensure that they were physically normal externally and were subsequently observed at least twice each day. Any changes in behaviour or clinical condition were recorded daily during the study.

Bodyweight: The bodyweight of each animal was recorded on arrival and on days 4, 7-16 (inclusive) and on days 19 and 22 of gestation.

Food consumption: The amount of food consumed by each animal over three day periods was measured by giving a weighed quantity of food contained in a glass jar on days 1, 4, 7, 10, 13, 16 and 19 and calculating the amount consumed from the residue on days 4, 7, 10, 13, 16, 19 and 22, respectively.

Terminal investigations: One rat requiring euthanasia was killed by over-exposure to halothane Ph. Eur. vapour and given a macroscopic examination *post mortem*.

On day 22 of gestation the animals were killed by over-exposure to halothane-Ph. Eur. vapour and a macroscopic examination *post mortem* was performed. The uterus from any animal without clear evidence of implantation was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred.

For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries and uterus were then examined and the following data recorded:-

Number of *corpora lutea* in each ovary

Number and position of implantations subdivided into:

- a) live foetuses
- b) early intra-uterine deaths (decidual or placental tissue only)
- c) late intra-uterine deaths (embryonic/foetal tissue plus placental tissue)

Individual foetal weights

The implantations were assigned letters of the alphabet to identify their position *in utero* starting at the ovarian end of the left horn and ending at the ovarian end of the right horn. In addition, each foetus was weighed and individually identified within the litter by means of a cardboard tag. After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 mL of 200 mg/mL pentobarbitone sodium solution.

Percentage pre-implantation loss and percentage post-implantation loss were calculated.

$$\% \text{ pre-implantation loss} = \frac{\text{number of } \textit{corpora lutea} - \text{number of implantations}}{\text{number of } \textit{corpora lutea}} \times 100$$

$$\% \text{ post-implantation loss} = \frac{\text{number of implantations} - \text{number of live foetuses}}{\text{number of implantations}} \times 100$$

Foetal observations: An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral

abnormalities, sexed, eviscerated and fixed in 70 % industrial methylated spirits. After approximately 24 hours the head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70 % industrial methylated spirits for subsequent processing and staining with Alizarin Red S. The stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed. The individual bones of the *manus* and *pes* were assessed and the result converted to a six point scale.

The observations were classified as major (permanent structural or functional deviations considered likely to be incompatible with survival or rarely seen) or minor defects or variants (small, generally transient deviations considered compatible with survival). The difference between the minor defect and variant classification is the frequency of occurrence in the control population of rats of this strain.

Statistical analyses: Data relating to animals which were non-pregnant, totally resorbed their litters or died intercurrently were excluded from the statistical analysis.

Maternal body weight during the dosing and post-dosing periods was considered by analysis of covariance on initial (day 7) body weight.

Maternal food consumption during the dosing and post-dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean *manus* and *pes* scores per litter were considered by analysis of variance.

Maternal-performance data (excluding the animal with undetermined pregnancy status), the proportion of foetuses with each individual *manus* and *pes* score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher's Exact Test.

Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external/visceral defects, minor external/visceral defects, external/visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:

All analyses were carried out in *SAS* (1989). For Fisher's Exact Test the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis.

All statistical tests were two-sided.

Results

Maternal toxicity:

Mortality and clinical signs: One control animal was killed on day 7 as a result of being misdosed. Excess watery fluid in the thoracic cavity and dark red areas on the surface of the

lung lobes were observed at examination *post mortem*. The pregnancy status of the animal was not determined.

There were no changes in the clinical condition of the animals given glyphosate acid which were considered to be treatment-related.

Body weight: There was no effect of glyphosate acid on maternal body weight.

Table B.6.6-23: Intergroup comparison of maternal body weight (g) (selected timepoints, adjusted means for days 8 and 22)

	Dose level of glyphosate acid (mg/kg/day)			
day	0 (control)	250	500	1000
1	255.6	255.5	253.5	252.8
8	288.2	288.1	288.0	287.5
22	406.4	410.1	411.1	408.6

Food consumption: There was no adverse effect of glyphosate acid on maternal food consumption. The amount of food consumed by the animals given 1000 mg glyphosate acid/kg/day was marginally lower during the dosing period but differences from the controls were not statistically significant.

Table B.6.6-24: Intergroup comparison of food consumption (g/day) (selected timepoints)

	Dose level of glyphosate acid (mg/kg/day)			
day	0 (control)	250	500	1000
1-4	23.9	24.6	24.6	23.2
13-16	33.2	33.4	33.7	31.9
19-22	29.5	31.6*	30.5	30.5

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

Sacrifice and pathology:

Gross pathology: There were no macroscopic findings which were considered to be related to the administration of glyphosate acid.

Developmental Toxicity: There was no effect of glyphosate acid on the number, growth or survival of the foetuses *in utero*.

Table B.6.6-25: Intergroup comparison of maternal performance

Observation	Glyphosate acid (mg/kg/day)			
	0 (control)	250	500	1000
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant	22	24	23	24
#pregnancy status not determined (intercurrent death)	1	0	0	0
Gravid uterus weight (g)	89.7	87.2	91.3	89.9
# Intercurrent deaths	0	0	0	0
# aborted	0	0	0	0
# totally resorbed at termination	0	0	0	1
<i>Corpora Lutea</i> /Dam	15.8	15.7	15.5	15.5
Implantations/Dam	14.4	12.9*	14.1	13.6
Total # Litters (viable)	22	24	23	23
Live Foetuses/Dam	12.9	12.4	13.1	12.9
Early (Proportion of litters affected)	8.7	3.4**	6.2	5.5
Late (Proportion of litters affected)	1.3	0.5	1.6	0.3
Litter Weight (g)	62.4	61.2	64.3	63.6
Mean Foetal Weight (g)	4.86	5.02	4.95	4.96
Sex Ratio (% Males per litter)	51.9	54.1	53.3	51.0
Preimplantation Loss (%)	8.7	18.0**	8.8	12.0
Postimplantation Loss (%)	9.9	4.9**	7.8	5.8*

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

Major defects: 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 glyphosate acid/kg/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The defects were dissimilar in type and of single incidence.

Minor defect: The proportion of foetuses with minor external/visceral defects and the proportion of foetuses with minor skeletal defects were similar for all groups. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.

Variants: The proportion of foetuses with external/visceral variants and the proportion of foetuses with skeletal variants were lower in the glyphosate acid treated groups than in the control group. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.

Manus and pes assessment: There was no effect of glyphosate acid on the ossification of the *manus* or *pes*.

Conclusion by the Notifiers

The dose level of 1000 mg glyphosate acid/kg/day was the no observed effect level in this study for both maternal and developmental effects.

Comment by RMS:

The study is considered acceptable. The conclusion is agreed.

Reference: IIA, 5.6.10/02
Report: [REDACTED] (1995)
 HR-001: Teratogenicity Study in Rats.
 [REDACTED]
 Data owner: Arysta LifeScience
 Study No.: IET 94-0152
 Date: 1995-07-21
 not published
 ASB2012-11497
Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985
 U.S. EPA FIFRA Guidelines Subdivision F, 1984
Deviations: None
GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 1995-03-23 to 1995-06-26

Materials and methods

Test material: Glyphosate technical
 Identification: HR-001
 Description: Solid crystals
 Lot/Batch #: 940908
 Purity: 95.68 %
 Stability of test compound: Not mentioned in the report
 Vehicle and/
 or positive control: Diet
 Test animals:
 Species: Rat
 Strain: SPF Crj:CD (SD)
 Source: Charles River Japan, Inc.
 Age: 13 weeks
 Sex: Males and females
 Weight at dosing: Males: 380 – 450 g, females: 267 – 322 g
 Acclimation period: 11 days
 Diet/Food: Certified diet MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*
 Water: Filtered and sterilized water, *ad libitum*
 Housing: By pair in aluminium cages with wire-mesh floors for mating period; Individually for copulated females in aluminium cages with wire-mesh floors.
 Environmental conditions: Temperature: 24 ± 2 °C
 Humidity: 55 ± 10 %
 Air changes: 12/hour
 12 hours light/dark cycle

Study design and methods:

In life dates: 1995-03-23 to 1995-06-26

Animal assignment and treatment:

Vaginal smears were taken from females for microscopic examination. Females showing proestrus or estrus vaginal smears were paired overnight with males on a 1:1 basis. The females were examined next morning for the presence of vaginal plugs and sperm in vaginal smears and considered to copulate when vaginal plugs and/or sperm were observed. These mating procedures were repeated for 4 consecutive days.

Four test groups were set. The test substance was administered orally with a stomach tube to 10 copulated Crj:CD (SD) female rats per group at dose levels of 0, 300, 1000 mg/kg/day from day 6 to 15 of gestation.

Clinical observations

Each female was observed for clinical signs and mortality at least once daily during the pre-dosing and post-dosing periods and at least twice daily during the dosing period.

Body weight

Individual body weights were recorded on days 0, 6-15 (daily during the dosing period) and 20 of gestation. Adjusted body weight gains were calculated by subtracting the gravid uterine weight from the body weight value on day 20 of gestation.

Sacrifice and pathology

All surviving females were euthanized by overdosage of ether inhalation and cesarian section was performed on day 20 of gestation. Each female was necropsied. The ovaries and uterus were removed and the gravid uterine weight and numbers of corpora lutea and implants were recorded. Then the uterus was opened and the numbers of live and dead foetuses were recorded with their positions in the uterine horns. Resorbed embryos or dead foetuses were classified into implantation sites, placental remnants or macerated foetuses (including dead foetuses) according to developmental stage in which resorptions or deaths occurred. When no uterine implants were grossly apparent, the uterus was stained with 10 % ammonium sulphide solution to detect very early resorptions. The weights of each live foetus and of each placenta were determined and recorded. Live foetuses were sexed and were euthanized by an intraperitoneal injection of pentobarbital sodium solution for examination of external abnormalities. The eyes were examined for alterations after removing the palpebral skin. Then the foetuses were examined for visceral and skeletal abnormalities.

Statistics

Variance analysis using Bartlett's test was evaluated for body weights, adjusted body weights, body weight gains and food consumption of maternal rats, numbers of corpora lutea, implants and live foetuses, and weights of gravid uteri, foetuses and placentas.

Results and discussion

Clinical observations

During the pre-dosing period, clinical observation revealed no abnormalities in any groups.

During the dosing period, no abnormalities were observed in maternal rats of the control group. In the 30 and 300 mg/kg groups, one or two maternal rats had hair loss or scabs on the skin which have been usually observed in the historical control rats. In the 1 000 mg/kg group, 20 out of 22 pregnant females showed slightly loose stool and the increase in its incidence was statistically significant.

During the post-dosing period, slightly loose stool was also observed on the following day of last dosing (day 16 of gestation) in 9 out of 20 females that showed this finding during the dosing period in the 1 000 mg/kg group. Another finding observed during this period was hair loss in 1-2 maternal rats in each treated group.

No deaths occurred during the study in any groups.

Body weight

No significant differences were found in the mean body weights and the mean adjusted body weights of maternal rats between the control groups and any of the treated groups.

No significant differences were found in the mean body weight gains of maternal rats between the control group and any of the treated groups.

Food consumption and compound intake

No significant differences were found in the mean food consumption of maternal rats between the control group and any of the 30 and 300 mg/kg groups. In the 1 000 mg/kg group, lower and higher values were observed in the mean food consumption at intervals of days 6-9 of gestation (early dosing period) and days 15-20 of gestation (post-dosing period), respectively, and the differences from the corresponding controls were statistically significant.

Necropsy:

Gross pathology at cesarian section

Gross pathological examination of maternal rats at cesarean section revealed several findings such as hair loss and pelvic dilatation in the kidney in 1-2 animals in all groups including the control group. These findings were not considered to be due to test substance treatment.

Ovaries and uterus

Out of 24 copulated females, 23, 24, 24 and 22 were proved to be pregnant in the control, 30 mg/kg, 300 mg/kg and 1 000 mg/kg groups, respectively.

No significant differences were found in the mean gravid uterine weights and the mean numbers of corpora lutea and implants between the control group and any of the treated groups.

Foetuses

Number of live foetuses and percent incidences of resorptions and foetal deaths

There were no significant differences in the mean number of live foetuses and the mean percent incidence of resorptions and foetal deaths between the control group and any of the treated groups.

Sex ratio, fetal body weights and placental weights.

There were no significant differences in the foetal sex ratio, the mean foetal body weights and the mean placental weights between the control group and any of the treated groups.

Findings in external, visceral and skeletal examination

External malformations observed were short tail in a foetus of the 30 mg/kg group and microphthalmia in a foetus of the 1 000 mg/kg group.

Visceral examination revealed two types of malformations: right aortic arch in a foetus of the 300 mg/kg group and ventricular septal defects in a foetus of each of the 300 and 1 000 mg/kg groups.

Visceral variations were observed in all groups including the control group. The types and number in fetuses were thymic remnant in the neck, dilatation of the renal pelvis and left umbilical artery in 16-26, 1-2 and 0-3, respectively.

Skeletal examination revealed three types of malformations: splitting of the ossification centers of the thoracic vertebral bodies in 2, 1 and 2 fetuses in the control, 300 mg/kg and 1 000 mg/kg groups, respectively, asymmetry of the sternbrae with sterno-costal joint displacement in a foetus of the 300 mg/kg group, and fusion of the sternbrae in a foetus of the 300 mg/kg group, and fusion of the sternbrae in a foetus of the 1 000 mg/kg group.

Skeletal variations were observed in all groups including the control group. The types and the number in fetuses were cervical ribs shortening of the 13th ribs, lumber ribs, sacralization of the lumber vertebra and asymmetry and/or splitting of the sternbrae in 0-1, 0-1, 1-11, 0-1 and 3-5, respectively

Conclusion by the Notifiers

Based on these results, no observable effect level and minimal toxic level in the teratogenicity study with technical glyphosate in SD rats were established as follows.

	Maternal rats	Foetal rat
No observable effect level	300 mg/kg/day	1000 mg/kg/day
Minimal toxic level	1000 mg/kg/day	-

It is also concluded that the highest dose level of 1000 mg/kg/day of HR-001 is not teratogenic to SD rat fetuses.

Comment by RMS:

The study is considered acceptable. The evaluation regarding maternal toxicity is agreed, which was confined to loose stool at highest dose level. The NOAEL for developmental toxicity is not supported due to the slight increase in skeletal variations at highest dose level: lumbar ribs were observed in 11 fetuses out of 7 litters compared to only 4 fetuses out of 2 litters in control animals. Teratogenic effects were not observed. Based on findings in dams and fetuses, the NOAEL for both maternal and developmental toxicity is considered to be 300 mg/kg bw/d.

B.6.6.11 Teratogenicity test by the oral route in the rabbit

Five developmental studies in rabbits had been submitted for the previous EU evaluation of which four may be still used following re-evaluation by the RMS. For the current re-evaluation the feeding study in rabbits by [REDACTED] (1981, TOX9650160) was considered not acceptable due to serious reporting deficiencies and therefore not reported in the present RAR.

In addition, three new studies in rabbits were provided in the GTF dossier. These studies were submitted either for the first time for this evaluation or had been subject to JMPR evaluation in 2004 yet and were all considered acceptable by the RMS.

Reference: IIA, 5.6.11/01
Report: [REDACTED] (1995)
 HR-001: A Teratogenicity Study in Rabbits
 [REDACTED]
 [REDACTED]
 Data owner: Arysta Life Sciences
 Study no.: [REDACTED] 94-0153
 Date: 1995-07-21
 not published
 ASB2012-11498
Guidelines: OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1995-03-31 to 1995-06-09

Materials and methods

Test material:
 Identification: Glyphosate technical, Code: HR-001
 Description: White crystal
 Lot/Batch #: T-941209
 Purity: 97.56 %
 Stability of test compound: Not reported
 Vehicle and/or positive control: 0.5 % carboxymethylcellulose
 Test animals:
 Species: Rabbit
 Strain: Japanese White rabbits Kbl:JW, SPF
 Source: Minowa Breeding Center, KITAYAMA LABES Co., Ltd
 Age: 18 weeks (females); 5-50 month (males)
 Sex: Males and females
 Weight at dosing: 3.32 – 4.08 kg
 Acclimation period: 10 days
 Diet/Food: GC4 (Oriental Yeast Co., Ltd.), *ad libitum* (females) / 120 g/day (males)
 Water: Tap water, *ad libitum*
 Housing: Individually in aluminium cages with wire-mesh floors.
 Environmental conditions: Temperature: 22 ± 2 °C
 Humidity: 55 ± 10 %
 Air changes: 15/hour
 12 hours light/dark cycle

Study design and methods:

In life dates: 1995-03-31 to 1995-06-09

Animal assignment and treatment:

In a teratogenicity study groups of 18 Japanese White female rabbits received doses of 0, 10, 100 and 300 mg/kg bw/day test substance in carboxymethylcellulose by gavage from Gestation Day 6-18 after artificial insemination performed on 12 or 16 females each day for 5 consecutive days. The dose levels were chosen based on results of a preliminary teratogenicity study.

Diet preparation and analyses

For each dose level, dosing solutions were prepared two times during the study by suspending the test substance in purified water with the aid of 0.5 % sodium carboxymethylcellulose. For each dose level dosing solutions were analyzed for concentration of the test substance before use.

Clinical observations

A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight

Individual body weights were recorded on Day 0, 6-18, 24 and 27 of gestation. Body weight gains were calculated by subtracting the body weight value on Day 0 of gestation from each value determined on Days 6 through 27 of gestation. Adjusted weights were also calculated by subtracting the gravid uterine weight from the body weight on Day 27 of gestation.

Food consumption

Food consumption of females was determined on alternate days from Day 0 to Day 26 of gestation and on Days 26- 27 of gestation. In each interval, daily food consumption (g/rabbit/day) was calculated for each female by dividing values of total food consumption by the number of days.

Sacrifice and pathology

Females were euthanased by an injection of an overdose of a pentobarbital sodium solution into the auricular vein on Day 27 of gestation and subjected to caesarean sectioning.

The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. When uterine implants were not grossly apparent, the uteri were stained with 10 % ammonium sulfide solution to detect very early resorptions. After examination of the ovaries and conceptuses, each female was necropsied.

Developmental parameters

Live foetuses and their placentas were individually weighed. Live foetuses were uniquely identified by litters. Then they were euthanatized by an intraperitoneal injection of a pentobarbital sodium solution and examined for external abnormalities. The eyes were examined for alterations after removing the palpebral skin. The sex of the foetuses was determined by observation of the gonads.

After these examinations, each foetus was examined for visceral abnormalities. Then the thoracic and abdominal organs were removed and preserved in 10 % neutral- buffered formalin along with the ovaries and placentas. The remaining skeletons were fixed in 70 % isopropanol, stained with alizarin red S and cleared in 70 % glycerin for examination of skeletal abnormalities. After examination, skeletal specimens were stored.

Statistics

The following statistical tests were used to estimate significance of differences between the control group and the treated groups. The data on body weights, adjusted body weights, body weight gains, and food consumption of maternal rabbits, numbers of corpora lutea, implants, and live foetuses, and weights of gravid uteri, foetuses and placentas were evaluated as follows: Equality of variances was first evaluated by Bartlett's test. When group variances were homogeneous, a parametric analysis of variance in one-way classifications was used to determine if any statistical differences exist among groups. If the analysis of variance was significant, Dunnett's t-test or Scheffé's multiple comparison test was performed to detect any statistically significant differences between the treated groups and their corresponding controls. When Bartlett's test indicated that the variances were not homogeneous, Kruskal-Wallis test was used for detecting any statistical differences among groups and if significant, Dunnett-type mean rank test or Scheffé-type mean rank test was performed to detect statistical differences between the treated groups and their corresponding controls. Fisher's exact probability test was used for the data on the incidences of clinical and gross pathological findings in maternal rabbits, incidences of maternal rabbits having foetuses with malformations and variations, incidences of foetal malformations and variations, and foetal sex ratio, and Mann-Whitney's U-test for the data on the percent incidences of resorptions and foetal deaths.

Results and discussion

Analysis of dose formulations

The test substance was detected at levels of 95-105 % of the target concentrations in each dosing solution.

Food consumption

Mean food consumption in the treated groups was comparable to that in the control group throughout the study period.

Mortality

One rabbit in the high dose group died on Day 20 of gestation without showing any clinical signs.

Clinical observations

During the treatment period one animal each showed hair loss (forelimb) and scab on the auricle, respectively in low and mid dose group, respectively (see Table B.6.6-26). In the high dose group four animals showed loose stool and two showed soiled fur in the perianal region that was considered to be an alteration caused by defecation of loose stool. The incidence of loose stool was significantly high when compared with the control.

During the post-dosing period, two and one animal in the control group showed loose stool and red material on the tray, respectively. In the low dose group, hair loss (forelimb) was found in one animal and loose stool in another. Besides these findings, one dam aborted on Day 20 of gestation, and another one prematurely delivered on Day 27 of gestation. In the mid dose group only one animal showed hair loss in the lower abdominal region. In the high

dose group, two animals out of four, that had shown loose stool during the dosing period, still showed this alteration, and one animal out of these two aborted on Day 26 of gestation. Although loose stool disappeared from the two other dams, the first prematurely delivered on Day 27 of gestation and the second had hair loss (dorsal region).

Considering the results of the preliminary study, defecation of loose stool and subsequent abortion or premature delivery observed in the highest dose group were considered to be related to test substance treatment.

Table B.6.6-26: Observed clinical signs during the dosing period

Clinical sign	Number of rabbits affected in dose group [#]			
	Control (0 mg/kg/day)	Low (10 mg/kg/day)	Mid (100 mg/kg/day)	High (300 mg/kg/day)
No abnormalities detected	18/18 (0)	16/17 (1)	15/16 (2)	13/17 (0)
Hair loss	0/18 (0)	1/17 (0)	0/16 (0)	0/17 (0)
Scab on the auricle	0/18 (0)	0/17 (0)	1/16 (0)	0/17 (0)
Soiled fur in the perianal region	0/18 (0)	0/17 (0)	0/16 (0)	2/17 (0)
Loose stool	0/18 (0)	0/17 (0)	0/16 (0)	4/17 (1)*

[#] x/y: number affected / total number of animals in group

* Significantly different from control at $p < 0.05$.

Figures in parentheses represent the number of animals having no grossly observable conceptus. These animals were excluded from statistical evaluation.

Body weight

Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, although differences from controls were not statistically significant, the mean values on Days 16-24 of gestation were somewhat lower than those in the control group.

Pathology

Necropsy

Necropsy of maternal animals aborted, prematurely delivered or found dead on the study noted no abnormalities in the rabbits in the low dose group. In the high dose group, the aborted rabbit had yellow-coloured adipose tissue, a hair bolus in the stomach, watery contents in the large intestine and accentuated lobular pattern in the liver. The prematurely delivered rabbit in the high dose group had soiled fur in the perianal region, erosion in the stomach, a hair bolus in the stomach, and watery contents in the caecum. In the dead rabbit, pale liver and ascites (red) in the abdominal cavity were found; however, the cause of death was not known.

Gross pathological findings observed in animals which survived to termination of the study were: hair loss in the lower abdominal or dorsal region in one animal in each of the mid and high dose groups; hair bolus in the stomach in one animal each of the control and low dose groups. The occurrence of these gross pathological findings was low, and considered to be unrelated to test substance treatment.

Observations on the ovary and uterus

In the control, low, mid and high dose groups, 18, 16, 18, and 15 females, respectively, survived to termination of the study and were proven to be pregnant. However, one, two and one females in the low, mid and high dose group, respectively, had no grossly observable conceptus while implantation sites were detected by uterine staining with a 10 % ammonium

sulfide solution, indicating very early resorptions; all data from these females were excluded from subsequent calculations.

Examinations of uterine contents demonstrated no abnormalities in all groups including the control. Mean gravid uterine weights and mean numbers of corpora lutea and implants were comparable between the control and the treated groups.

Developmental parameters

Number and viability of fetuses

No statistically significant differences were noted in the mean number of live fetuses and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

Sex ratio, foetal body weights and placental weights

No statistically significant differences were noted in the sex ratios, mean foetal body weights, mean placental weights, mean number of live fetuses, and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

External, visceral and skeletal examination

No statistically significant differences were noted in the incidences of maternal animals having fetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the number of litters with malformations was significantly higher than that in the control group (see Table B.6.6-27). This increased malformation rate was due to an increase in skeletal malformations, as no external or visceral malformations were noted in fetuses from the high dose group. This was considered to be a sporadic alteration rather than the test substance treatment-related alteration because the types of skeletal malformations observed were inconsistent. Further, a dose-response in the number of fetuses showing skeletal malformations was not evident across dose groups.

With regard to variations, the incidence of total no. of litters with skeletal variations in the 100 mg/kg bw/day group was significantly higher than that in the control group. This high value was due to a significantly high incidence (87.5 % of litters, 27.3 % of the fetuses) of lumbar ribs in this dose group when compared with the control (72.2 % of litters, 16.4 % of fetuses). The total litter incidence for skeletal variations in the 100 mg/kg/day group was 100 %. However, the increased incidence of lumbar ribs in the 100 mg/kg/day group was considered to be a sporadic alteration because the value was within the historical control range (8.1-35.0 % of examined fetuses), and because no such increase was observed in the 300 mg/kg bw/day group (13.4 %).

Table B.6.6-27: Incidence of fetal malformations and variations in rabbits treated with HR-001

Foetal findings	Dose level (mg/kg bw/day)			
	0	10	100	300
Malformations				
No. of litters examined	18	15	16	14
No. of foetuses examined	140	130	150	112
No of litters with anomalous foetuses	1	3	3	5*
Percentage of litters with malformations (%)	5.5	20.0	18.8	35.7
Skeletal malformations				
Fusion of the frontal/parietal bones	0	1	0	2
Fissure of the parietal bone	0	0	3	0
Hypoplasia of the interparietal bone	0	1	0	0
Splitting of the parietal bones	0	0	3	1
Shortening of the nasal/frontal/mandibular bones	0	0	1	0
Hemivertebra	1	0	0	2
Unilateral ossification centre of the thoracic/lumbar vertebral bodies	0	1	0	0
Bifurcation of the ribs	1	0	0	0
Sternal cleft	0	0	1	0
Splitting of the sternbrae with sternocostal joint displacement	0	2	0	0
Total no. of foetuses with skeletal malformations	1	4	6	5
Percentage of foetuses with skeletal malformations (%)	0.7	3.1	4.0	4.5
Total no. of litters with skeletal malformations	1	3	2	5
Percentage of litters with skeletal malformations (%)	5.6	20.0	12.5	29.4
Variations				
No. of litters examined	18	15	16	14
No. of foetuses examined	140	130	150	112
No of litters with anomalous foetuses	16	14	16	8*
Percentage of litters with variations (%)	88.9	93.3	100	57.1
Skeletal variations				
No. of foetuses examined	140	130	150	112
27 presacral vertebrae	4	1	4	3
27 presacral vertebrae with 13 th ribs	12	9	15	12
Cervical ribs	1	3	1	1
Lumbar ribs	23	19	41*	15
Extra ossification centre anterior to the 1 st sternebra with costal cartilage joining	0	0	0	1
Total no. of foetuses with skeletal variations	40	32	61*	31
Total no. of litters with skeletal variations	16	12	16	8
Percentage of litters with skeletal variations (%)	88.9	80	100	57.1

* Significantly different from control at $p < 0.05$.**Conclusion by the Notifiers**

The oral administration of HR-001 to artificially inseminated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 300 mg/kg bw/day. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 100 mg/kg bw/day for maternal toxicity. The NOAEL for offspring was considered to be 300 mg/kg bw/day.

Comment by RMS:

The study is considered acceptable. The NOAEL for both maternal and developmental toxicity in this study conducted with artificial inseminated rabbits are agreed. No developmental toxic effects were observed.

Reference: IIA, 5.6.11/02
Report: [REDACTED] (1996)
Glyphosate technical: Oral gavage teratology study in the rabbit
[REDACTED]
Data owner: Nufarm
SPL project no.: 434/020
Date: 1996-07-04
GLP: yes
not published
ASB2012-11499
Guidelines: OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations: None
GLP:
Acceptability: *See RMS comment*

Dates of experimental work: 1995-10-13 - 1995-12-12

Materials and methods

Test material:
Identification: Glyphosate technical
Description: White powder
Lot/Batch #: H95D161A
Purity: 95.3 %
Stability of test compound: not reported
Vehicle and/or positive control: 1 % carboxymethyl cellulose
Test animals:
Species: Rabbit
Strain: New Zealand White
Source: Charles River (UK) Ltd., Margate, Kent, UK
Age: 17 - 19 weeks
Sex: Females (time-mated)
Weight at dosing: 2.2 - 4.1 kg
Acclimation period: At least 4 days
Diet/Food: SQC Standard Rabbit Diet (SDS Ltd., Witham, Essex, UK), *ad libitum*
Water: Tap water, *ad libitum*
Housing: Individually in stainless steel cages with grid floor
Environmental conditions: Temperature: 20 ± 3 °C
Humidity: 50 ± 20 %
Air changes: 15/hour
12 hours light/dark cycle

Study design and methods:

In life dates: 1995-10-13 - 1995-12-12

Animal assignment and treatment in the preliminary study:

Twenty-four time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated Day 0 of gestation. The females were delivered to Safepharm Laboratories Ltd. at or before Day 3 of gestation and were allocated randomised to treatment groups. Groups of 6 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1 % carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation Day 7-19. The dose levels were chosen based on results of a preliminary dose finding study with 6 female nulliparous rabbits, where administration of 500 or 1000 mg/kg bw resulted in toxicity signs (scours, fluid filled caecum, stomach ulceration, body weight loss, reduced food consumption). Based on these findings dose levels of ≥ 500 mg/kg bw were considered to be too high for a prolonged study.

Animal assignment and treatment in the main study:

Seventy-two time-mated females were supplied as described for the preliminary study (see above). Groups of 18 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1 % carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation Day 7-19.

Dose formulation and analysis

For each dose level, the test material was suspended daily in 1% carboxymethyl cellulose by weighing the required amount into a glass jar and adding vehicle to make the appropriate final volume. Homogeneity was assured by mixing the formulation with a homogeniser. The concentration, stability and homogeneity of the test material were analysed. The formulation was stable for at least 1 h.

Clinical observations

A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight

Individual body weights were recorded on Day 3, 7, 10, 13, 16, 19, 22, 25 and 29 of gestation.

Food consumption

Food consumption of females was recorded on Days 3 to 7, Days 7 to 10, Days 10-13, Days 13-16, Days 16-19, Days 19-22, Days 22-25 and Days 25-29 of gestation.

Sacrifice and pathology

Females were euthanatized by an i.v. injection of an overdose of sodium pentobarbitone into the auricular vein on Day 29 of gestation, examined for macroscopic abnormalities and subjected to caesarean sectioning. The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. After examination of the ovaries and conceptuses, each female was necropsied.

Developmental parameters

The foetuses were killed by intrathoracic injection of sodium pentobarbitone. All foetuses were dissected and examined for visceral abnormalities macroscopically. The heads of alternate foetuses were removed and identified using an indelible marker and placed in Bouin's fixative. After a minimum of 14 days, the heads were transferred to 90 % industrial methylated spirits (IMS) in distilled water and examined for visceral anomalies under a low power binocular microscope. All foetuses were identified using colour coded wires and placed in 70 % IMS in distilled water. The foetuses were eviscerated, processed and the skeletons stained with alizarin red. The foetuses were examined for skeletal development and anomalies.

Statistics in the main study

Female bodyweight change (relative to Day 7 of gestation) and food consumption were analysed statistically by one-way analysis of variance with the Bonferroni multiple comparison test followed by pair wise analysis of control values against treated group values using Students 't' test where appropriate.

All foetal parameters, skeletal development, group incidence of specific visceral and skeletal anomalies were analysed statistically by Kruskal-Wallis non parametric analysis of variance followed by pair wise analysis of control values against treated values using the Mann-Whitney U - test where appropriate.

Results and discussion

Analysis of dose formulations

The test substance was detected at the levels of 81-102% of the target concentrations in each dosing solution.

Food consumption

In the preliminary study, significantly reduced food consumption was observed while administering in the high dose level of 400 mg/kg/day (Days 7 to 19 of gestation). This observation was confirmed in the main study. At the high dose level, there was a reduction in food consumption during the dosing period compared to controls (Days 10 to 13, $p < 0.05$; Days 13 to 19, $p < 0.01$). No other significant changes were observed in the remaining groups during the main study.

Mortality

In the preliminary study, two does were killed in extremis in the high dose group, one had aborted foetuses and the other was bleeding from the vagina. No mortalities occurred at any dose up to 400 mg/kg/day in the preliminary study.

In the main study, two rabbits were found dead or moribund at the high dose level. One female was found dead prior to dosing on Day 19 of treatment. One female was killed *in extremis* on Day 20 of treatment. Clinical observations noted at this time included hunched posture, lethargy, ptosis, hypothermia and blood on the litter tray. At the intermediate dose level, one female was found dead after dosing on Day 16 of treatment. Necropsy findings of reddened lungs, a fluid filled thorax and test material in thoracic cavity are consistent with mal-dosing. At the low dose level, no mortalities occurred. One female was found dead two minutes after dosing in the control group. Necropsy findings of blood in thorax, inflated appearance of lungs and a large area of congestion on the right caudal lobe are consistent with mal-dosing.

Clinical Observations

In both the preliminary and the main study, the clinical signs were in general the same. There was a toxicologically significant increase in the incidence of clinical observations, particularly scours, reduced faecal output and diarrhoea at the high dose level (400 mg/kg bw/day). Observations of lethargy, ptosis, hunched posture, hypothermia and blood on tray were noted for one animal of the main study killed *in extremis*.

At 200 mg/kg bw/day, vaginal bleeding and blood on tray were noted for one animal of the main study. Scours were also noted in animals at 200 and 50 mg/kg bw/day as well as in the control group, but the incidence and duration were not as severe as at the high dose level (see Table B.6.6-28). No other treatment-related observations were evident.

Thus, for the findings observed at doses below 400 mg/kg bw/day, a clear dose-response could not be established.

Table B.6.6-28: Observed clinical signs during the dosing period

Clinical sign	Number of rabbits affected in dose group [#]			
	Control (0 mg/kg/day)	Low (50 mg/kg/day)	Intermediate (200 mg/kg/day)	High (400 mg/kg/day)
Scours	5/14 (4)	10/18 (0)	7/16 (2)	16/16 (2)
Reduced faecal output	0/14 (4)	1/18 (0)	2/16 (2)	2/16 (2)
Diarrhoea	0/14 (4)	1/18 (0)	0/16 (2)	10/16 (2)
Diuresis	0/14 (4)	0/18 (0)	1/16 (2)	0/16 (2)
Blood on tray	0/14 (4)	0/18 (0)	1/16 (2)	1/16 (2)
Noisy respiration	0/14 (4)	0/18 (0)	1/16 (2)	1/16 (2)
Lethargy	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Ptosis	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Hunched posture	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Hypothermia	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Anal staining	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Subdued behaviour	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Vaginal bleeding	0/14 (4)	0/18 (0)	1/16 (2)	0/16 (2)

[#] x/y: number affected / total number of animals in group

Figures in parentheses represent the number of animals having no grossly observable conceptus.

Body weight

In the preliminary study a toxicologically significant decrease in body weight gain from Day 13 to 19 *post coitum* was evident at the high and intermediate dose levels.

Likewise a reduction in group mean body weight gain from Days 9 to 29 *post coitum* was observed in the high dose level group during the main study. The difference in group mean bodyweight change compared to controls was statistically significant ($P < 0.05$ to 0.01) from Days 13 to 29 *post coitum*. Also in the intermediate dose level group a slight reduction (although not statistically significant) in group mean body weight gain from Day 9 to Day 29 *post coitum* was noted. In the low dose level group body weight gain was comparable to controls throughout the study period (see Table B.6.6-29).

Table B.6.6-29: Mean body weight gain during gestation

Dose level (mg/kg bw)	No. of animals	Body weight change (g) at Day (relative to Day 7)						
		10	13	16	19	22	25	29
0 (Control)	14	29	95	202	260	314	375	409
50	18	12	75	158	223	278	325	395
200	15	-11	54	143	198	263	309	294
400	15	-33	-45*	11**	21**	96**	153**	250*

* Significantly different from control at $p < 0.05$.

** Significantly different from control at $p < 0.01$.

Pathology

Necropsy

The macroscopic necropsy findings of the two does of the high-level dose group that died or were killed *in extremis* included fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, duodenum congested and colon, rectum and appendix gas distended. These findings indicate that the test material may affect the gastrointestinal tract. The animal killed *in extremis* at this level also had both uterine horns containing blood and dead fetuses in the uterus. This may be a result of maternal toxicity. All other necropsy findings were not treatment-related.

Observations on the ovary and uterus

No treatment related effects were evident in both the preliminary and the main study.

In the control, low, intermediate and high dose level groups 14, 18, 16, and 16 females, respectively, survived to termination of the main study and were proven to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.

Developmental parameters

Number and viability of foetuses

The litter size at caesarean section was comparable in all treatment groups. In the high dose level group, there were slight but not statistically significant, increases in late foetal deaths and post implantation loss, mainly due to one animal that had nine late deaths, resulting in a post implantation loss of 69.2 %. This was therefore considered not to be a treatment-related effect. At 200 mg/kg bw/day, there were statistically significant increases ($p < 0.05$) in total foetal deaths and post implantation loss. These increases were caused by a slight, but not statistically significant, rise in early foetal deaths. As at this dose level, there was no rise in late foetal deaths, as seen at the high level; the effect on early foetal deaths was considered not to be treatment-related.

Foetal body weights

No statistically significant differences were noted in the mean foetal body weights between the control group and the treated groups. Mean total litter weights were comparable in all treatment groups.

External, visceral and skeletal examination

At the high dose level, there was one litter with one foetus with major malformations. This foetus was found to have spina bifida and clubbed and malrotated hind limbs. At the intermediate dose level, two foetuses of two different litters had major malformations. One

foetus had retinal infolding and a haemorrhage in the retinal layer, the other acephaly, small kinked tail, bilateral forelimb flexure, interrupted aorta and an intraventricular septal defect. At skeletal examination, this foetus was found to have multiple rib and vertebral column abnormalities. At the low dose level, three foetuses of two different litters had major abnormalities. In one litter, one foetus had forked ribs with a displaced vertebral centrum. In another litter, one foetus had a small eye with retinal infolding and aphakia. A second foetus from this litter had nostrils close together, and a thin nasal septum not attached at posterior pole near the front of the nasal passages. In the control group, there were two foetuses from two different litters with major abnormalities. One foetus had gastroschisis and the other foetus had an extra vertebral arch resulting in scoliosis.

These findings were considered to be within the range of normal variation for this species. There were no treatment-related effects on the degree of skeletal development.

Table B.6.6-30: Incidence of foetal malformations and variations in rabbits treated with glyphosate acid

Foetal findings	Dose level (mg/kg bw/day)			
	0	50	200	400
No. of litters examined	14	18	15	15
No. of foetuses examined	128	157	119	134
Skeletal malformations				
Total no. of foetuses with skeletal malformations	1	0	1	0
Total no. of litters with skeletal malformations	1	0	1	0
Percentage of litters with skeletal malformations (%)	7.1	0.0	6.7	0.0
Skeletal variations				
Total no. of foetuses with skeletal variations	43	48	39	49
Total no. of litters with skeletal variations	13	18	15	15
Percentage of litters with skeletal variations (%)	92.8	100	100	100
External and visceral findings				
No. of litters examined	14	18	15	15
No. of foetuses examined	128	157	119	134
No of litters with anomalous foetuses	2	5	2	3
Percentage of litters with anomalous foetuses (%)	14.3	27.8	13.3	20
No. of litters with major malformations	2	2	2	1
Percentage of litters with malformed foetuses (%)	14.3	11.1	13.3	6.7

Conclusion by the Notifiers

The oral administration of glyphosate technical to pregnant rabbits by gavage from gestation Day 7-19 resulted maternal toxicity at 400 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 200 mg/kg bw/day for maternal toxicity. The 'No Observed Adverse Effect Level' (NOAEL) for developmental toxicity was considered to be 400 mg/kg bw/day.

Comment by RMS:

The study is considered acceptable. The NOAEL of 200 mg/kg bw/d for maternal toxicity is not supported. The NOAEL is considered to be 50 mg/kg bw/d due to slight reduction in body weight gain at 200 mg/kg bw/d.

The NOAEL of 400 mg/kg bw/d for developmental toxicity is neither supported. The NOAEL is considered to be 50 mg/kg bw/d due to significantly increased post-implantation loss at 200 mg/kg bw/d. The statement, that this increase was caused by a slight rise in early foetal deaths and not in late foetal deaths, as seen at the high dose level and therefore considered not to be treatment-related, cannot be followed, because there is no information given

regarding the mechanism behind this foetal deaths. Due to some reporting deficiencies, it remains unclear, whether the heart was part of visceral examination.

Comment by GTF on the first draft of the RAR (July 2013):

1. The GTF believes the developmental NOAEL in Coles and Doleman is 400 mg/kg/day, with no evidence to test substance related dose-response, citing the published expert review: Kimmel et al., 2013, (ASB2013-3462) Evaluation of developmental toxicity studies of glyphosate with attention to cardiovascular development, *Crit Rev Toxicol*, 2013, 43(2): 79–95.

2. Regarding [REDACTED] (1996, ASB2012-11499) the RMS notes that “it remains unclear whether the heart was part of visceral examination”. The study sponsor contacted the contract laboratory and the response is noted....The contract laboratory Head of reprotox at the contract notes Reprotox “The examination of the heart is conducted on the fresh fetus for rabbit developmental toxicity studies. This is mentioned in our standard operating procedure“. This is reflected the draft RAR Volume 3, B.6.6.11, noting “All foetuses were dissected and examined for visceral abnormalities macroscopically”

RMS comment (August 2013):

1. As already stated, the NOAEL is considered to be 50 mg/kg bw/d due to significantly increased post-implantation loss at 200 mg/kg bw/d. The statement, that this increase was caused by a slight rise in early foetal deaths and not in late foetal deaths, as seen at the high dose level and therefore considered not to be treatment-related, cannot be followed, because there is no information given regarding the mechanism behind this foetal deaths (please refer to the additionally inserted table below. Considering the individual animal data: at low dose level of 50 mg/kg bw/d 4/18 pregnant animals revealed post implantation losses, which was comparable to control animals (4/14 animals). Whereas at the mid dose of 200 mg/kg bw/d 10/15 animals were affected and at 400 mg/kg bw/d 9/15 animals. Currently, RMS does not see the need to change the assessment.

[Remark: The published expert review by Kimmel et al. (2013, ASB2013-3462) was already available and considered when preparing the first draft of the RAR, please refer to B.6.6.12-Published data]

Table B.6.6-31: Group Mean Litter Data

Group	Dose Level (mg/kg)	Number of Animals	Number Pregnant at Necropsy		Number of Corpora Lutea	Number of Live Foetuses	Total Number of Implants	% Male Foetuses	Embryonic/Foetal Deaths			Implantation Loss %		Total Litter Weight (g)	Mean Foetal Weight (g)
									Early	Late	Total	Pre	Post		
1	0	18	14	Mean	10.9	9.1	9.5	56.2	0.21	0.14	0.36	12.5	3.7	372.0	41.5
				SD	2.2	2.5	2.5	10.5	0.43	0.53	0.63	18.2	6.5	92.6	5.5
2	50	18	18	Mean	10.5	8.7	9.1	51.0	0.22	0.11	0.33	13.6	3.6	334.6	39.4
				SD	2.4	2.4	2.3	11.4	0.55	0.32	0.77	9.4	8.5	75.1	5.6
3	200	18	15	Mean	10.7	7.9	8.9	53.9	0.87	0.13	1.00*	16.4	11.5*	321.5	41.7
				SD	2.1	2.5	2.5	11.3	1.06	0.35	1.00	15.5	11.4	79.2	4.5
4	400	18	15	Mean	11.5	8.9	10.3	50.8	0.47	0.93	1.40	9.3	12.1	337.0	38.2
				SD	1.8	2.6	2.3	11.7	0.92	2.28	2.35	12.5	18.6	84.4	5.2

SD = standard deviations

* = significantly different from control value (p < 0.05)

2: The additional information is acceptable, but was not given in the study report.

Reference: IIA, 5.6.11/03
Report: [REDACTED] (1996)
 Glyphosate acid: Developmental toxicity study in the rabbit
 [REDACTED]
 Data owner: Syngenta
 Report No.: CTL/P/5009
 Date: 1996-07-02
 not published
 TOX2000-2002
Guidelines: OECD 414 (1981), EEC B.31 (1988), US-EPA 83-3
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1996-01-01 to 1996-02-09

Materials and methods

Test material:
 Identification: Glyphosate acid
 Description: White solid
 Lot/Batch #: Y04704/034
 Purity: 95.6 %
 Stability of test compound: The stability of the test substance was confirmed for the study period.
 Vehicle and/or positive control: Deionised water
 Test animals:
 Species: Rabbit
 Strain: New Zealand White
 Source: Harlan Interfauna Ltd., Huntingdon, Cambridgeshire, UK
 Age: Not reported
 Sex: Females (time-mated)
 Weight at dosing: approximately 3.8 kg
 Acclimation period: At least 4 days
 Diet/Food: Harlan Teklad 9603TRB rabbit diet, *ad libitum*
 Water: Tap water, *ad libitum*
 Housing: Individually in mobile rabbit units
 Environmental conditions: Temperature: 17 ± 2 °C
 Humidity: 55 ± 15 %
 Air changes: 25-30/hour
 12 hours light/dark cycle

Study design and methods

In life dates: 1996-01-01 to 1996-02-09

Animal assignment and treatment:

Eighty time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated Day 1 of gestation. The females were delivered to CTL at or before Day 3 of gestation and were allocated randomised to treatment groups. Groups of 20 time-mated New Zealand white female rabbits received 0, 100, 175 or 300 mg/kg bw/day test substance by gavage (2 mL/kg bw) from gestation Day 8-20. The dose levels were chosen based on results of a preliminary dose finding.

Dose formulation and analysis

For each dose level an appropriate amount of deionised water was added to a weighed amount of glyphosate acid (adjusted for purity). Each preparation was thoroughly mixed and subdivided into aliquots. Fresh aliquots were used for each day of the study. Two preparations were made per concentration (i.e. 0, 50, 87.5 and 150 mg/mL). The dosing preparations were stored at room temperature.

Representative samples of each dosing preparation were analysed prior to being used for dosing to verify the achieved concentration of glyphosate acid in the vehicle. Samples were taken for the determination of homogeneity at 50 and 150 mg glyphosate acid/mL (low and high dose levels).

The chemical stability of glyphosate acid in the vehicle was determined by re-analysis of the lowest and highest concentrations of the dosing preparations after an interval of 40 days.

Dose formulations were shaken prior to dosing, and during dosing as required.

Clinical observations

A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight

Individual body weights were recorded on arrival, on Day 4, prior to dosing on Days 8 to 20 and on Days 23, 26 and 30 of gestation.

Food consumption

Food consumption of females was recorded on Days 4-8, Days 8-11, Days 11-14, Days 14-17, Days 17-20, Days 20-23, Days 23-26 and Days 26-30 of gestation.

Sacrifice and pathology

All rabbits at scheduled termination on day 30 and any requiring euthanasia during the study were killed by an overdose of 200 mg/mL sodium pentobarbitone solution given as i.v. injection. All animals were subjected to an examination post mortem. This involved an external observation and an examination of the thoracic and abdominal viscera. The pregnancy status of each animal was determined. Where there was no clear evidence of implantation, the uterus was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred. For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries, uterus and contents were then examined. Number of corpora lutea, number and position of implantations, number of live foetuses, foetus weight and early and late intrauterine deaths were determined for each sacrificed doe.

Developmental parameters

After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 mL of 200 mg/mL pentobarbitone sodium solution. An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral abnormalities, sexed, eviscerated and fixed in 70 % industrial methylated spirits. After approximately 24 h the head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70 % industrial methylated spirits for subsequent processing and staining with Alizarin Red S. The remaining stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed.

Statistics

Data relating to those animals which were non-pregnant and animals that died intercurrently were excluded from the statistical analysis. Maternal bodyweight during the dosing and post dosing periods was considered by analysis of covariance on initial (Day 8) bodyweight. Maternal food consumption during the dosing and post dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean *manus* and *pes* scores per litter were considered by ANOVA. Maternal performance data, the proportion of foetuses with each individual *manus* and *pes* score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher's Exact Test. Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external visceral defects, minor external/visceral defects, external visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:

- 1) Percentages were analysed by ANOVA following double arcsine transformations,
- 2) the proportion of foetuses and, with the exception of male foetuses, the proportion of litters affected were considered by Fisher's Exact Test.

All analyses were carried out in SAS (1989). For Fisher's Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis.

All statistical tests were two sided.

Results and discussion

Analysis of dose formulations

The concentrations of glyphosate acid in the dosing formulations were within 12 % of the target concentrations. The homogeneity and stability of the test substance in the dosing formulations was satisfactory.

Food consumption

During the dosing period, does receiving 175 or 300 mg/kg bw/day showed reduced food consumption compared to the controls.

Mortality

The incidence of intercurrent deaths was 1, 2, 2 and 2 in the control, 100, 175 and 300 mg/kg bw/day groups, respectively.

In the post-dosing period, one doe in the control group showed weight loss, reduced food consumption, signs of diarrhoea, mucus in the faeces, few faeces and staining in the genital area. This animal aborted on Day 30. Changes in the stomach and caecum were observed post mortem.

In the low dose level group, one doe showed slight loss of bodyweight and reduced food consumption between Days 4 and 8 (i.e. prior to the onset of dosing) and this response continued into the dosing period, until the animal aborted its litter on Day 19. Examination post mortem noted the presence of a mass in the right inguinal region of the abdominal cavity. A second animal in this group aborted its litter on Day 25 having shown weight loss and reduced food consumption from Day 14.

At the intermediate dose level, one doe was killed for humane reasons on Day 23 having shown loss of bodyweight and reduced food consumption from Day 4 on. By Day 23, the animal had become thin and subdued and all uterine implantations were found to be dead. A second animal in this group aborted its litter on Day 22 having shown slight weight loss from Day 14 and reduced food consumption from Day 4.

At the high dose level, two animals aborted their litters on Days 24 and 23, respectively. Both animals showed a reduction in food consumption from Day 11 and bodyweight loss from Day 11/13. A hair-like substance was found in the stomachs of both animals at examination post mortem.

Clinical Observations

In the high dose level group, there was an increased incidence of animals producing few faeces, with signs of diarrhoea or with staining in the genital area, in comparison with the control group. The production of few faeces and signs of diarrhoea were also of increased incidence in does of the intermediate dose group. There were no clinical effects observed in rabbits treated at a dose level of 100 mg/kg bw/day (see Table B.6.6-32).

Table B.6.6-32: Observed clinical signs during the dosing period

Clinical sign	Number of rabbits affected in dose group			
	Control (0 mg/kg/day)	Low (100 mg/kg/day)	Intermediate (175 mg/kg/day)	High (300 mg/kg/day)
Blood on tray	0	2	2	1
Cold	0	0	1	0
Dry sores 1 or more areas	0	1	0	0
Ears torn	0	2	1	1
Eye opaque	0	1	0	0
Few faeces on tray	3	3	9	9
Mucus in faeces	1	0	0	0
No faeces on tray	0	1	2	3
Scabs in 1 or more areas	4	6	3	3
Signs of diarrhoea	4	5	11	19
Staining in genital area	2	2	3	11
Subdued behaviour	0	0	1	0
Thin	0	0	1	2
Urine coloured	0	1	1	0
Wet sores in 1 or more areas	2	0	1	0

Body weight

Administration of 300 mg/kg bw/day was associated with a reduction in maternal body weight gain. The statistical different observed body weight development at 175 mg/kg bw/day was due to differences in body weights at the begin o the study. All animals except one of the high dose group showed signs of recovery in the post-dosing period. The reduction in food consumption was therefore accompanied by a corresponding reduction in body weight. In the low dose level group, body weight gain was comparable to controls throughout the study period (see Table B.6.6-33).

Table B.6.6-33: Mean body weight development (in g) during gestation

		Dose level in mg/kg bw/day			
		0 (Control)	100	175	300
Animals per group		17	18	17	17
Day of gestation	8	3924	3771	3822	3815
	9	3845	3837	3834	3823
	10	3857	3863	3856	3830
	11	3885	3873	3874	3854
	12	3894	3879	3877	3856
	13	3917	3905	3902	3880
	14	3942	3932	3930	3875
	15	3975	3982	3939	3896
	16	4020	4031	3959	3907*
	17	4049	4053	3982	3923*
	18	4063	4051	3990	3914**
	19	4085	4061	4005	3927**
	20	4088	4059	3995	3926**
	23	4177	4118	4049*	3951**
	26	4236	4210	4169	4093*
	30	4313	4294	4256	4183

* Significantly different from control at $p < 0.05$.

** Significantly different from control at $p < 0.01$.

Pathology

Necropsy

There were no macroscopic findings that were considered to be related to the administration of glyphosate acid.

Observations on the ovary and uterus

No treatment related effects were evident in the study.

In the control, low, intermediate and high dose level groups 17, 18, 17, and 17 females, respectively, survived to termination of the main study and were proven to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.

Developmental parameters

Number and viability of foetuses

The proportion of foetuses that were male was statistically significantly increased in the intermediate dose level group, in comparison with the control group. In the absence of a dose-related trend, this finding was considered incidental to the administration of glyphosate acid.

There was no adverse effect of glyphosate acid on the number or survival of the fetuses in utero.

Foetal body weights

There was a statistically significant reduction in mean foetal weight in the high dose level group, in comparison with the control group. This difference was considered attributable to two litters for which the mean pup weight was particularly low.

External, visceral and skeletal examination

The number of fetuses with major defects was 3/143 (2/17 litters), 1/147 (1/18 litters), 0/135 (0/17 litters) and 2/144 (2/17 litters) in the control, 100, 175 and 300 mg/kg bw/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The proportion of fetuses with minor external/visceral defects was similar for all groups, including the control. There were no significant differences in litter incidences for minor external/visceral defects noted. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid (see Table B.6.6-34 and Table B.6.6-35).

The proportion of fetuses with minor skeletal defects was statistically significantly increased in the 100 and 300 mg/kg bw/day groups, in comparison with the control group, but not in the 175 mg/kg bw/day group. Evaluation of the specific defects noted an increased incidence of fetuses in the high dose level group with partially ossified transverse processes on the 7th cervical vertebra (8 fetuses in 2 litters), unossified transverse processes on the 7th lumbar vertebra (14 fetuses in 4 litters) or partially ossified 6th sternebra (16 fetuses in 7 litters). None of the specific minor defects were statistically significantly increased in the low or intermediate dose level groups. None of the fetuses were found to have an external/visceral variant.

The proportion of fetuses with skeletal variants was statistically significantly increased in the high dose level group, in comparison with the control group. Evaluation of the specific variants noted a slight, but not statistically significant, increase in the incidence of fetuses in this group with partially ossified odontoids (62 fetuses in 15 litters) or with 27 pre-sacral vertebrae (37 fetuses in 12 litters).

The slightly higher mean *manus* score observed in the high dose level group, in comparison with the control group, was due to a slight reduction in ossification as shown by the increase in incidence of fetuses scoring 4 or 5. A similar response was apparent from the *pes* scores.

Table B.6.6-34: Summary of the type and incidence of major defects

Major foetal defects	Number of foetuses affected in dose group*			
	Control	Low	Intermediate	High
	(0 mg/kg/day)	(100 mg/kg/day)	(175 mg/kg/day)	(300 mg/kg/day)
Heart single ventricle, ventricle walls thickened, aorta enlarged, pulmonary artery reduced	0/143	1/147	0/135	1/144
Encephalocoele (gross malformation of the skull)	0/143	0/147	0/135	1/144
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, aorta enlarged, persistent truncus arteriosus	1/143	0/147	0/135	0/144
Shortened upper and lower jaws, cleft lip, cleft palate, nares absent, forepaws flexed (right extremely, left slight)	1/143	0/147	0/135	0/144
Reduced number of lumbar vertebrae (25 pre-sacral vertebrae)	1/143	0/147	0/135	0/144

* number affected / total number

Table B.6.6-35: Summary of the type and incidence of major defects (litter incidences)

Major foetal defects	Number of litters affected in dose group*			
	Control	Low	Intermediate	High
	(0 mg/kg/day)	(100 mg/kg/day)	(175 mg/kg/day)	(300 mg/kg/day)
Heart single ventricle	0/17	1/18	0/17	1/18
aorta enlarged	1/17	1/18	0/17	1/18
pulmonary artery reduced	0/17	1/18	0/17	1/18
Encephalocoele (gross malformation of the skull)	0/17	0/18	0/17	1/18
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, Shortened upper and lower jaws, cleft lip, cleft palate, nares absent	1/17	0/18	0/17	0/18
persistent truncus arteriosus	1/17	0/18	0/17	0/18
forepaws flexed (right extremely, left slight)	1/17	0/18	0/17	0/18
Reduced number of lumbar vertebrae, 25 pre-sacral vertebrae	1/17	0/18	0/17	0/18

* number affected / total number

Table B.6.6-36: Incidence of foetal malformations and variations in rabbits treated with glyphosate acid

Foetal findings	Dose level (mg/kg bw/day)			
	0	100	175	300
No. of litters examined	17	18	17	17
No. of foetuses examined	143	147	135	144
Skeletal malformations				
Total no. of foetuses with major defects	3	0	0	1
Total no. of litters with major defects	2	0	0	1
Percentage of litters with major defects (%)	11.8	0.0	0.0	5.9
Total no. of foetuses with minor defects	58	82*	59	79*
Total no. of litters with minor defects	16	18	16	17
Percentage of litters with minor defects (%)	94.1	100	94.1	100
Skeletal variations				
Total no. of foetuses affected	119	129	116	132*
Total no. of litters affected	17	18	17	17
Percentage of litters affected (%)	100	100	100	100
External and visceral findings				
No. of foetuses with major defects	2	1	0	2
No of litters with foetuses with major defects	2	1	0	2
Percentage of litters with foetuses with major defects (%)	11.8	5.6	0.0	11.8
No. of foetuses with minor defects	12	7	9	11
No of litters with foetuses with minor defects	8	5	8	7
Percentage of litters with foetuses with minor defects (%)	47.1	27.8	47.1	41.2

* Statistically significant from control ($p < 0.05$)

Conclusion by the Notifiers

The oral administration of glyphosate acid to time-mated rabbits by gavage at a maximum dose level of 300 mg/kg bw/day from Gestation Day 8-20 resulted maternal toxicity at 175 and 300 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore the 'No Observed Effect Level' (NOEL) was considered to be 100 mg/kg bw/day for maternal toxicity. The 'No Observed Effect Level' (NOEL) for developmental toxicity was considered to be 175 mg/kg bw/day.

Comment by RMS

The study is considered acceptable. The NOAEL for both maternal and developmental toxicity are agreed. Maternal toxicity comprised clinical signs, reduced food consumption and body weight gain at 175 mg/kg bw/d and above. Developmental toxicity consisted of reduced foetal body weight and reduced ossification at 300 mg/kg bw/d.

Reference:

IIA, 5.6.11/04

Report:

(1980)
Technical Glyphosate: Teratology study in rabbits

Monsanto Report No.: IR-79-016

Date: 1980-02-29

not published

TOX9552392

Guidelines:	Not stated. (pre-guideline; satisfies in general the requirements of OECD 414 (1981), but not of OECD 414 (2001))
Deviations:	Not applicable
GLP:	no (pre-GLP study)
Acceptability:	<i>See RMS comment</i>

Dates of experimental work: 1979-04-10 to 1979-05-11

Materials and methods

Test material:

Identification: Glyphosate technical

Description: White powder

Lot/Batch #: XHJ-64

Purity: 98.7 %

Stability of test compound: Not reported

Vehicle and/
or positive control: 0.5 % aqueous Methocel®

Test animals:

Species: Rabbit

Strain: Dutch Belted

Source:

Age: Approx. 7 month

Sex: Females

Weight at dosing: 2.533 – 3.234 kg

Acclimation period: At least 30 days

Diet/Food: Purina Rabbit Chow Checkers 5301, *ad libitum*

Water: Tap water, *ad libitum*

Housing: Individually in suspended wire mesh cages

Environmental conditions: Temperature: Exact values not reported

Humidity: Exact values not reported

Air changes: Exact values not reported

Light controlled

Study design and methods

In life dates: 1979-04-10 to 1979-05-11

Animal assignment and treatment:

Sixty-four female Dutch Belted rabbits were artificially inseminated and randomly assigned to treatment groups of 16 animals. The day of insemination was designated Day 0 of gestation. The rabbits received daily doses of 0, 75, 175 or 350 mg/kg bw/day test substance by gavage (1 mL/kg bw) from gestation Day 6 to 27. Individual doses based on individual body weights determined on gestation Day 6.

Dose formulation

For each dose level an appropriate amount of grounded technical glyphosate was suspended in 0.5 % aqueous Methocel® solution and homogenised. The dose solutions were prepared daily.

Clinical observations

A check for mortality or behavioural changes was made once daily prior to treatment. During the treatment and post-treatment period all rabbits were observed once daily for clinical signs of toxicity, mortality or behavioural changes.

Body weight

Individual body weights of dams were recorded on gestation Days 0, 6, 12, 18, 24 and 28. These time points for body weight determination differ from the requirements of the current OECD guideline 414 (2001) (i.e., body weights should be determined on gestation day 0 and at 3-day intervals thereafter). Although the time-intervals were longer than required, the time points for body weight determination are considered to be sufficient to evaluate the body weight development of the pregnant animals.

Sacrifice and pathology

Dams

All rabbits at scheduled termination on day 28 were sacrificed, the uterus was excised and weight and the foetuses were removed. The number and location of viable foetuses, early and late resorptions, the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs were examined for gross pathological changes. Rabbits that died during the study were necropsied to determine the cause of death.

Foetuses

All foetuses were weight, sexed and examined for external malformation and variations, as well as for visceral malformations and variations. The carcasses were then fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for skeletal examination.

Statistics

All statistical analyses compared the treatment groups to the control group with a level of significance at $p < 0.05$. Foetal sex distribution and number of litters with malformations were analysed using the Chi-square test with Yates correction and/or Fisher's exact probability test. The number of early and late resorption and post-implantation losses were compared by the Mann-Whitney U-test. Mean numbers of viable fetuses, total implantations, corpora lutea and mean fetal body weights were compared by ANOVA (one-way), Bartlett's test for homogeneity and appropriate t-test.

Results and discussion

Mortality

There was an increased incidence of mortalities in the high dose group (see Table B.6.6-37).

Table B.6.6-37: Mortalities of dams

	Control (0 mg/kg/day)	Low (75 mg/kg/day)	Intermediate (175 mg/kg/day)	High (350 mg/kg/day)
Spontaneous deaths*	0/16	1/16	2/16	10/17
Time of death (gestation day)	--	26	22, 25	3 to 21
% mortality	0.0	6.3	12.5	58.8
Sacrificed after abortion	2	0	1	1
Sacrificed on gestation day	22	--	27	23

* dead animals / total animals in group

For five of the rabbits that died spontaneously, the cause of death was attributed to pneumonia, respiratory disease, enteritis or gastroenteritis. For one rabbit of the mid-dose group and the other 7 rabbits of the high dose group, the cause of death could not be determined.

The mortality rates in the intermediate- and especially in the high-dose groups were greater than 10 %, which exceeds the OECD guideline 414 (2001) suggestion of no more than approximately 10 % maternal mortality.

Clinical Observations

Clinical signs consisting of soft stool and diarrhea were noted in all dose groups during the treatment period. In the 175 mg/kg bw/day dose group, the incidence of this finding was slightly increased when compared with the control group. At 350 mg/kg bw/day, either soft stool, diarrhea or both were observed in each animal at least once during the treatment period. Also in the high dose group, there was an increased incidence of animals with nasal discharge in comparison with the control group.

Body weight

There were no treatment-related effects on maternal body weights and body weight gain.

Pathology

Necropsy

There were no macroscopic findings in dams that were considered to be related to the administration of glyphosate technical.

Observations on the ovary and uterus

No treatment-related effects were evident in the study.

Table B.6.6-38: Maternal observations

	Historical control	Control (0 mg/kg/day)	Low (75 mg/kg/day)	Intermediate (175 mg/kg/day)	High (350 mg/kg/day)
Surviving dams at caesarean section*	27/28	14/16	15/16	13/16	6/17
Pregnant rabbits	24/28	12/16	15/16	11/16	6/17
Non-pregnant rabbits	3/28	2/16	0/16	2/16	0/17
Abortions	1/28	2/16	0/16	1/16	1/16

* number of surviving animals / total animals in group

Developmental parameters

There were no statistically significant differences in the mean numbers of early or late resorptions, total implantations, corpora lutea, foetal body weights or foetal sex ratio in any of

the test substance groups when compared to control. The number of viable foetuses was slightly, but statistically significantly, increased in the low-dose group at 75 mg/kg bw/day. However, this finding was considered incidental and not related to the test substance.

The mean foetal body weights were slightly decreased in the test substance groups as compared to control. However, the mean foetal body weights in all test substance groups were comparable to the historical control data (i.e. 30.9 g) (see table below).

Table B.6.6-39: Mean litter data at caesarean section

	Historical control	Control (0 mg/kg/day)	Low (75 mg/kg/day)	Intermediate (175 mg/kg/day)	High (350 mg/kg/day)
Pregnant dams [#]	24	12	15	11	6
Viable foetuses/dam	6.7	5.3 ± 2.73	7.6* ± 1.84	5.9 ± 2.77	6.3 ± 2.25
Post implantation loss/dam ^{##}	0.8	0.7 ± 0.89	0.4 ± 0.63	0.2 ± 0.40	0.8 ± 1.33
Total implantations /dam ^{##}	7.5	5.9 ± 2.39	8.0 ± 1.81	6.1 ± 2.84	7.2 ± 2.93
Corpora lutea/dam ^{##}	10.1	9.0 ± 2.13	10.1 ± 1.64	10.5 ± 3.45	8.5 ± 1.87
Foetal sex distribution (males/females) [#]	83/77	28/35	53/61	32/33	17/21
Mean foetal body weight (g) ^{##}	30.9	33.4 ± 7.27	30.9 ± 4.43	29.9 ± 7.21	29.3 ± 4.82

[#] Total number

^{##} Number ± SD; historical control without SD

* Statistically significant difference compared to control (p < 0.05)

It should be noted that, in all dose groups, the number of pregnant dams were less than the number of pregnant dams required by the current OECD guideline 414 (2001); i.e., 16. Therefore, the evaluation of the developmental parameters may be limited.

Skeletal and visceral examination

The percentages of foetuses with skeletal malformations were 0.0, 2.6, 3.1 and 5.3 in the control, 75, 175 and 350 mg/kg bw/day groups, respectively. Although malformations were observed in the test substance groups, neither the type nor incidence of the malformations provided evidence for an adverse effect of glyphosate acid. There were no visceral malformations observed in any of the dose groups including control. There were no statistically significance differences in the variation observed in the test substance group when compared to the control group (see Table B.6.6-40).

Table B.6.6-40: Summary of foetal malformations and variations

	Hist. contr.	Control (0 mg/kg/day)		Low (75 mg/kg/day)		Intermediate (175 mg/kg/day)		High (350 mg/kg/day)	
		x/y	%	x/y	%	x/y	%	x/y	%
<i>Number of litters examined</i>		12		15		11		6	
	%	x/y	%	x/y	%	x/y	%	x/y	%
<i>Skeletal malformations</i>		0/63	0.0	3/114	2.6	2/65	3.1	2/38	5.3
Exencephaly	--	0/63	0.0	0/114	0.0	1/65 (1/11)	1.5	0/38	0.0
Acrania	--	0/63	0.0	0/114	0.0	0/65	0.0	1/38 (1/6)	2.6
Scoliosis with associated rib anomalies	0.6	0/63	0.0	2/114 (2/15)	1.8	0/65	0.0	0/38	0.0
T1 rib absent	--	0/63	0.0	0/114	0.0	1/65 (1/11)	1.5	0/38	0.0
Carpal flexure	0.6	0/63	0.0	0/114	0.0	0/65	0.0	1/38 (1/6)	2.6
Fused cervical vertebral centra	0.6	0/63	0.0	1/114 (1/15)	0.9	0/65	0.0	0/38	0.0
<i>Visceral malformation</i>	--	0/63	0.0	0/114	0.0	0/65	0.0	0/38	0.0
Total malformations		0/63	0.0	3/114	2.6	2/65	3.1	2/38	5.3
<i>Variations</i>									
27 presceral vertebrae	8.7	6/63 (5/12)	9.5	7/114 (3/15)	6.1	9/65 (4/11)	13.8	7/38 (5/6)	18.4
13 th rudimentary rib(s)	3.7	5/63 (3/12)	7.9	14/114 (6/15)	12.3	3/65 (3/11)	4.6	3/38 (3/6)	7.9
13 th full rib(s)	8.1	3/63 (3/12)	4.8	10/114 (4/15)	8.8	5/65 (2/11)	7.7	6/38 (3/6)	15.8
Hyoid arches bent	--	--	--	2/114 (1/15)	1.8	1/65 (1/11)	1.5	--	--
Hyoid body unossified	--	6/63 (2/12)	9.5	2/114 (2/15)	1.8	6/65 (3/11)	9.2	--	--
Parietals reduced in ossification	0.6	1/63 (1/12)	1.6	--	--	1/65 (1/11)	1.5	--	--
Sternebrae #5 and/or #6 unossified	5.6	6/63 (3/12)	9.5	13/114 (7/15)	11.4	13/65 (5/11)	20.0	4/38 (2/6)	10.5
Pubis unossified	--	4/63 (1/12)	6.3	1/114 (1/15)	0.9	4/65 (1/11)	6.2	--	--
Talus unossified	--	3/63 (1/12)	4.8	--	--	5/65 (3/11)	7.7	--	--
Extra ossification center, cervical area	--	--	--	--	--	1/65 (1/11)	1.5	--	--
Major vessel variations	8.7	11/63 (6/12)	17.5	14/114 (8/15)	12.3	14/65 (5/11)	21.5	6/38 (4/6)	15.8

x/y: number of foetuses affected / total number of foetuses examined

(a/b): number of litters affected / total number of litters

Conclusion by the Notifiers

The oral administration of glyphosate acid to pregnant rabbits by gavage from Gestation Day 6-27 resulted maternal toxicity at ≥ 175 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore the NOAEL was considered to be 75 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 350 mg/kg bw/day.

Comment by RMS (Re-evaluation):

The study is considered supplementary, because the highest dose level revealed only 6 does with litters for examination due to the high maternal mortality. The NOAEL of 75 mg/kg bw/d for maternal toxicity is agreed. However, the previously established NOAEL for developmental toxicity of 350 mg/kg bw/day was lowered to 175 mg/kg bw/day because of the low number of available fetuses in the high dose group avoiding meaningful evaluation.

Comment by GTF on the first draft of the RAR (July 2013):

██████████ (1980, TOX9552390) is considered a supplementary study based on high maternal mortality and therefore an insufficient number of litters to thoroughly evaluate potential developmental effects at 350 mg/kg/day. However, the study data are still robust and applicable for establishing valid NOAEL values from the mid and low doses of 175 and 75 mg/kg/day, respectively.

Further explanation:

While a dose-response curve cannot be confirmed for adverse developmental endpoints, due to high maternal mortality and thus insufficient number of litters at the high dose, a dose-response is evident for maternal mortality and toxicity. Therefore this study may be considered robust for establishing the maternal toxicity NOAEL of 75 mg/kg/day. Similarly, the mid and low doses clearly demonstrated no adverse effects to offspring, and thus the offspring NOAEL of 175 mg/kg/day in this study is unequivocal, especially as this value was also the developmental NOAEL in the Moxon study.

RMS comment (August 2013):

What is the idea behind this comment? It is only a summary of the conclusion/comment given by the RMS in the Draft RAR.

Reference:

IIA, 5.6.11/05

Report:

██
(1991)
The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations)

██
Data owner: Cheminova
Study/Project No.: CHV 45 & 39 & 40/901303
Date: 1991-10-14
not published
TOX9552391

Guidelines:

OECD 414, US EPA 83-3

Deviations:

None

GLP:

Yes

Acceptability:

See RMS comment.

Dates of experimental work: 1989-12-14 to 1990-03-02

Materials and methods

Test material:

Identification: Glyphosate acid

Description: White solid

Lot/Batch #: 206-JAK-25-1

Purity: 98.6 %

Stability of test compound: Stable over the duration of the study

Vehicle and/
or positive control: 1 % methylcellulose

Test animals:

Species: Rabbit

Strain: New Zealand White

Source: Interfauna UK Ltd., Huntingdon, UK

Age: 11-24 weeks (on delivery)

Sex: Female

Weight at dosing: Females: 3582 – 3709 g (mean values)

Acclimation period: 7 days

Diet/Food: SQC Standard rabbit diet (Special Diet Services Ltd., Essex, UK), *ad libitum*

Water: Mains drinking water, *ad libitum*

Housing: Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage

Environmental conditions: Temperature: 19 ± 1 °C
Humidity: 49 ± 15 %
Air changes: Not recorded
Natural lighting supplemented with artificial lighting from 07 – 21:00 hours

Study design and methods:

In life dates: 14 -12-1989 – 02-03-1990

Animal assignment and treatment:

In a developmental toxicity study, groups of 16 - 20 time-mated female New Zealand White rabbits were administered glyphosate in 1% methylcellulose (dose volume 5 mL/kg) once daily by gavage at dose levels of 0 (vehicle control), 50, 150 or 450 mg/kg bw/day from Day 7 to Day 19 of pregnancy. Dose volumes were calculated for individual animals on day 7 and adjusted according to body weight on Days 9, 11 and 15. The day of mating was considered as Day 0. Dose levels were based on the findings of a preliminary study.

Dosing formulations were prepared daily and administered within 3 hours of preparation.

Observations

All animals were regularly handled and observed daily for overt changes or signs of reaction to treatment. Animals that died or were killed for animal welfare reasons were weighed and subjected to post-mortem examination.

Body weight

Individual body weights were recorded Days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation.

Food consumption and compound intake

Food consumption was recorded on days of weighing throughout gestation.

Sacrifice and pathology

On day 29 of pregnancy all surviving does were subjected to post-mortem examinations for congenital abnormalities and gross pathological changes in maternal organs.

The ovaries and uteri were examined to determine the number of corpora lutea, the number and distribution of live young, the number and distribution of embryonic and /foetal deaths, individual foetal weight and foetal abnormalities. Embryonic/foetal deaths were classified as Early, Late or Abortions.

Litter parameters

Live young were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were killed by intrathoracic injection of pentobarbitone sodium then weighed and dissected for examination of visceral abnormalities. Where appropriate, suspected abnormalities were further examined by alternative procedures such as microdissection and histopathology to clarify initial observations. Pups were fixed in industrial methylated spirit, the heads sliced along the line of the frontoparietal suture and the brain examined for abnormalities before clearing and staining by the modified Dawson technique of the carcasses for skeletal examination. Structural changes were presented as malformations, anomalies or variants.

Statistics

Two-tailed tests for significance were performed on litter data only, and significance at 1 % and 5 % were reported. Mean values of litter size, pre and post-implantation loss, litter weight, mean foetal weight and the incidence of anomalous offspring were analysed by the Kruskal-Wallis test. Intergroup comparisons were made by the non-parametric equivalent of the Williams' test following a significant h-statistic. Where 75 % of the values for a given variable consisted of one value, a Fisher's exact test was used.

Results and discussion

Analysis of dose formulations

The analysis of the dosing formulations taken at the first dosing showed the mid- and high-dose group to be within 6 % of the nominal dose whilst the low-dose group was 19 % below the nominal dose; however, a reanalysis on Day 19 showed the concentration to be 5 % above the nominal dose.

Food consumption

During the dosing period, females receiving 150 and 450 mg/kg bw/day showed reduced food consumption compared to the controls. A slight reduction was evident from Days 11 – 19 at 150 mg/kg bw/day (approximately 12 % compared with controls) and throughout the treatment period for the 450 mg/kg bw/day dose group (6-17 % during Days 7-19) (see Table B.6.6-41).

Table B.6.6-41: Summary of mean food consumption (g/rabbit/day)

	Dose Group (mg/kg bw/day)			
	0 (control)	50	150	450
Mated females	19	19	16	20
No. of animals included in assessment	18	12	15	13
Food consumption (g/rabbit/day) during				
Days 1-6	142	143	141	152
Days 7-8	143	154	150	135
Days 9-10	146	148	148	132
Days 11-14	153	149	134	129
Days 15-19	148	151	131	123
Days 20-23	142	154	149	149
Days 24-28	131	143	153	166

Mortality

There was one death in the 450 mg/kg bw/day dose group on Day 20 following signs of abortion on Day 19 and signs of gastrointestinal disturbance, manifested as soft/liquid faeces, severe reduction in food consumption and bodyweight loss from the onset of treatment. Two other deaths (a broken hind leg and an incidence of congenital abnormality) were unrelated to the treatment and were eliminated from the study assessment.

Clinical Observations

Clinical signs included a dose-related increase in the number of females showing soft/liquid faeces (gastrointestinal disturbances) and signs of lack of appetite (off feed/reduction in food consumption) at 150 and 450 mg/kg bw/day (see Table B.6.6-41).

Table B.6.6-42: Summary of relevant clinical signs in does

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	50	150	450
Mated females	19	19	16	20
Not pregnant	0	6	1	5
Number of does with live young or litters at Day 29	18	12	15	13
Clinical signs#				
Off-feed	8	6	10	9
Reduced faecal output	9	8	11	12
Soft/liquid faeces	0	2	5	13

Only animals with live young included

Body weight

A slight reduction in body weight gain was noted from Day 11 of pregnancy to termination of treatment in the 150 and 450 mg/kg bw/day dose groups, which coincided with the reduction in food consumption during the same period (see Table B.6.6-42).

Table B.6.6-43: Summary of body weight data (group means)

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	50	150	450
Mated females	19	19	16	20
No of animals included in assessment	18	12	15	13
Bodyweights (g) at				
Day 1	3538	3524	3568	3658
Day 7	3582	3604	3624	3709
Day 9	3589	3639	3637	3732
Day 11	3601	3653	3661	3743
Day 15	3742	3804	3779	3833
Day 20	3770	3831	3775	3835
Day 24	3844	3927	3849	3965
Day 29	3999	4084	3975	4103

Pathology**Necropsy**

Gross examination of does at post-mortem did not identify any treatment-related effects.

Observations on the ovary and uterus

A total of 18, 12, 15 and 13 pregnant females survived to termination and 163, 104, 112 and 95 fetuses were recorded for the 0 (control), 50, 150 and 450 mg/kg bw/day dose groups respectively. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss was recorded for one female of the 450 mg/kg bw/day dose group which aborted on Day 19 and died and also for one female at 50 mg/kg bw/day. One female at 150 mg/kg bw/day aborted 1/9 fetuses.

There were no significant intergroup differences in the numbers of corpora lutea, implantations, pre-implantation loss, foetal sex ratios or foetal weights (see Table B.6.6-44). There was a statistically significant increase in embryo/fetal death and post-implantation loss at all exposure levels. The study investigators questioned the biological significance of these findings for several reasons: 1) No dose-response pattern was evident, 2) the control value was at the lower end of the historical control range, while those of the exposed groups were at the higher end, and 3) the values in all groups were within or slightly above the historical control range. The latter two statements are supported by the historical control data provided in the study report (page 32) (see Table B.6.6-44). Although embryo/foetal death was within the historical control range, post-implantation loss was above the historical control values in the high-dose group, and both of these parameters were statistically significant ($p < 0.01$) at the high dose.

Table B.6.6-44: Summary of the maternal and litter parameters (group mean values)

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 does with live young or litters at Day 29	18	12	15	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 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	--

* 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 $P > 0.05$

Developmental parameters

Number and viability of foetuses

There were 18, 12, 15 and 13 viable litters at 0, 50, 150 and 450 mg/kg bw/day, respectively. The concurrent control showed low mean values for embryonic deaths and post implantation losses when compared with historical control values. When compared with these historical data as noted above, mean values in the treated groups were within the expected range; therefore, it was concluded that no adverse effect on foetal survival was attributed to glyphosate.

Foetal body weights

There was a dose-related reduction in mean foetal weight on a litter basis in all treated groups (not statistically significant) compared with the control; however, the mean individual foetal weight was not affected.

External, visceral and skeletal examination

Malformations were slightly increased in the 150 and 450 mg/kg bw/day dose groups compared to controls and appeared to be associated with an apparent increase in malformations of the thoracic region. However, neither the incidence nor the percentage of malformed foetuses was outside the historical control range and the values were not statistically different from concurrent control values. Several of the cardiovascular malformations that were observed, particularly in the high-dose group, occurred in the same animals and are related to a single morphogenetic mechanism (i.e., displacement of the developing aorticopulmonary septum), which is likely to adjust during the first two weeks of postnatal life. These related findings, which often cluster together, included dilated/narrow aorta and narrow/dilated pulmonary artery; interventricular septal defect; and disproportionately sized right and left ventricles. These findings were observed (often in clusters) in the historical control data that were provided by the conducting laboratory.

Individual presentation of these malformations in tables when the malformations occurred together in the same foetus and are due to the same mechanisms and artificially inflates the sense that there is a much stronger cardiac effect than is actually present.

The cardiac malformation observed with greatest frequency in this study was interventricular septal defect. The number of foetuses and litters with ventricular septal defects were 1, 1, 1 and 4 in the 0, 50, 150 and 450 mg/kg bw/day dose groups, respectively. Comparison of the historical control data shows that the heart findings (when presented on a percent individual and/or litter incidence basis) were slightly outside of the historical background range from 13 studies conducted during the same period. However, the disparity in values is a consequence of the small numbers of litters in the study report. If the data are displayed as a fraction (rather than a percentage), then the number of litters affected were 1/18, 1/12, 1/15, and 4/13 in the 0, 50, 150, and 450 mg/kg/day dose groups, respectively. The historical control range is 0/19 – 3/13. Thus, the findings at the high dose are barely outside of the historical control range. Further, they were observed in conjunction with clear signs of maternal toxicity (reduced food consumption, body weight gains and increased clinical signs).

The other cardiovascular finding found in this study not related to the morphogenetic mechanism involving formation of the spiral septum is retroesophageal right subclavian artery. This finding was also observed regularly throughout the historical period. It is not uncommon and is oftentimes an inconsequential anatomical difference in vascular arrangement. At autopsy this condition is found in 0.5 – 2.0 % of subjects.

The malformations of the cranial region, the lumbar and the lumbar/sacral regions did not show any treatment-related trend and are considered to be incidental. The incidences of anomalies and variants did not suggest any treatment relationship. The incidence of foetuses with reduced ossification did not show any dose-relationship; however, lower foetal weights were observed for the 450 mg/kg bw/day dose group with reduced ossification.

The observed foetal malformations and anomalies are summarised in the following (Table B.6.6-45).

Table B.6.6-45: Summary of foetal parameters

Parameter	Dose Group (mg/kg bw/day)				Historical control range or x/y \diamond (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
<u>Thoracic region malformations</u>					--
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
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	--

 \diamond 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

Conclusion by the Notifiers

Glyphosate was not teratogenic in this developmental toxicity study in rabbits. The NOAEL for maternal toxicity was 50 mg/kg bw/day based on clinical signs of toxicity including reduced feed consumption and body weight gain and soft/liquid faeces during the dosing

period. The NOAEL for foetotoxicity was 150 mg/kg bw/day based on statistically significantly increased embryo/foetal deaths and post-implantation loss. The NOAEL for teratogenicity was 450 mg/kg bw/day.

Comment by RMS (Re-evaluation):

The study is considered acceptable. In this study there was a significant increase in embryonic death and post-implantation loss in treated groups compared to controls, however without a clear dose-relationship. Regarding the post-implantation losses, values for the low and high dose groups are outside the historical control range.

The cardiac malformation observed with greatest frequency in this study was the interventricular septal defect. At 450 mg/kg bw/d this effect was outside the historical control range (4.2 % compared to 0.66 % in historical controls). Taken into account the high post-implantation loss at the same dose level, the incidence of additionally cardiac malformation may be covered, therefore. At mid dose level fetuses with an higher incidence of retro-oesophageal right subclavian artery were reported. However, this effect has to be considered equivocal, because no clear-dose relationship could be established.

In conclusion, the NOAEL for maternal toxicity is considered 50 mg/kg bw/d based on slightly restricted inappetence, slightly reduced body weight gain and soft/liquid faeces at 150 mg/kg bw/d. The NOAEL for developmental toxicity is considered to be 150 mg/kg bw/d based on the post-implantation loss, late embryonic death and an increase in cardiac malformations at 450 mg/kg bw/d.

Reference:

IIA, 5.6.11/06

Report:

[REDACTED] (1993)

Teratogenicity study in rabbits – Test compound: Glyphosate technical (FSG 03090 H/05 March 1990)

[REDACTED]

Data owner: Feinchemie Schwebda GmbH

Study No.: POXI: 884-TER-RB

Date: 1993-04-17, amended 1994-06-18

not published

TOX9551106

Guidelines:

OECD 414 (1981)

Deviations:

None

GLP:

Acceptability:

See RMS comment

Dates of experimental work: 1991-12-24 to 1992-03-06

Materials and methods

Test material:

Identification:

Glyphosate acid

Description:

Odourless white crystals

Lot/Batch #:

60

Purity:

96.8 %

Stability of test compound:

Stable over 2 years at ambient temperature.

Vehicle and/or positive control:	0.5 % w/v carboxymethylcellulose
Test animals:	
Species:	Rabbit
Strain:	Female New Zealand White
Source:	[REDACTED]
Age:	Approximately 6 months and above (at the start of study)
Sex:	Males and females
Weight at dosing:	Females: >2500 g (mean values)
Acclimation period:	At least 10 days.
Diet/Food:	Pelleted rabbit diet, supplied by M/S Lipton India Ltd, Bangalore (composition and feed analysis reports were provided) was provided <i>ad libitum</i>
Water:	Protected deep bore well drinking water, treated via activated charcoal filter and UV in Aquaguard on-line water filter-cum-purifier provided <i>ad libitum</i>
Housing:	Individually in 3-tier all aluminium cages with wire mesh bottom and common self-draining litter trays.
Environmental conditions:	Temperature: $22 \pm 3^{\circ}\text{C}$ Humidity: 40–70 % Air changes: 10–15/h Natural lighting supplemented with fluorescent lighting 12 hours light/dark cycle

Study design and methods:

In life dates: not reported

Animal assignment and treatment:

In a developmental toxicity study, groups of presumed mated female New Zealand White rabbits were administered once daily by gavage, glyphosate (batch no.: 60, purity 96.8 %) in 0.5 % carboxymethylcellulose (dose volume 2 mL/kg) at dose levels of 0 (vehicle control), 20, 100 or 500 mg/kg bw/day from Day 6 to Day 18 of pregnancy. Dosages for individual animals were calculated from day 6 and adjusted daily according to body weight. Dose levels were based on the findings of preliminary studies.

In a preliminary dose-range finding study, one male rabbit/dose group was administered by gavage glyphosate technical dissolved in 0.5 % carboxymethylcellulose (dose volume 2 mL/kg bw) at dose levels of 0 (control), 10, 20, 50, 500 or 1000 mg/kg bw/day for 13 days. Doses of ≥ 500 mg/kg bw/day resulted in loss in body weight and in feed intake and the 1000 mg/kg bw/day test animal died on Day 9 of treatment.

In a second dose-range finding study, one pregnant rabbit was administered 500 mg/kg bw/day glyphosate from day 6 to 18 of gestation and the findings compared with that of 20 historical control animals. Caesarean section and terminal necropsy was performed on day 28. There were no signs of toxicity from the treatment; body weight gain was greater (26 % more than the historical control mean) but notable apparent treatment-related changes were substantial reduction in feed intake (34 % of historical control mean) and reduced litter size in the test female (4) compared with the historical control mean (7).

Observations

All animals were observed twice daily for onset and duration of signs of toxicity and for mortality. All animals in the experiment that died, were killed moribund or killed at termination were subjected to post-mortem gross pathological examination. Tissues with gross lesions were preserved for histopathological examination as necessary.

Body weight

Individual body weights were recorded on Days 0, daily from Days 6 – 18 and on Day 28 of gestation.

Food consumption and compound intake

Food consumption was recorded on days of weighing throughout gestation.

Sacrifice and pathology

On day 28 of pregnancy, all surviving dams were subjected to post-mortem examinations and pups were delivered by Caesarean section. The ovaries and uteri were excised and weighed and maternal and foetal data were recorded. The maternal data determined were pregnant/non-pregnant, uterine weight, the number of corpora lutea, the number of implantations, the number of embryonic and foetal resorptions. The foetal data recorded were the number of dead/abnormal/live foetuses, individual foetal weight and sex.

Litter parameters

All the foetuses were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were euthanised with ether and visceral organs examined by a modified Wilson technique. Skeletal assessments were performed after appropriate preparation including staining in Alizarin Red. Structural changes were presented as variants, minor and major malformations.

Statistics

Statistical methods employed included the following. Maternal body weight and weight gain, feed intake, number of corpora lutea, number of implantations and mean foetal weight were analyzed by Bartlett's test followed by ANOVA and Dunnett's test. Day '0' and absolute body weight data were compared by the Paired Student's 't' test. The number and percent embryonic resorptions and foetal resorptions, the number of dead foetuses, the number of abnormal foetuses and percentage pre-implantation and post-implantation loss by Mann Whitney test. Litter size was by Student 't' test. The sex ratio, number of dams with any resorptions, number of dams with all resorptions and incidence of malformations were analysed by Chi-square test.

The statistical analysis and comparison of individual treatment groups with control value were done at 5 % probability level and the results were designated as significantly higher (+) / lower (-) than control value at $P \leq 0.05$.

Results and discussion

Analysis of dose formulations

Not reported.

Food consumption

During the dosing period, feed consumption was significantly reduced (31 %) in females receiving the 500 mg/kg bw/day dose compared to the controls (see Table B.6.6-46). Feed consumption during the post-treatment period did not show significant intergroup differences.

Table B.6.6-46: Summary of food consumption

Parameter	Historical positive control#	Dose Group (mg/kg bw/day)			
		0 (control)	20	100	500
<u>Food consumption (g/rabbit/day)</u>					
No of dams included in assessment	7	20	13	12	6
Day 0 – 6 (Pre-treatment)	105	114	88*	125	118
Day 6 – 19 (Treatment)	70*	103	109	102	71*
Day 19 – 28 (Post treatment)	129	109	135	107	105
Day 0 - 28	96	107	113	108	92

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw (treatment: Day 6-18; post-treatment: Day 18-28)

* Significantly lower than controls by Dunnett's test $P \leq 0.05$

Mortality

The four and eight deaths observed in the mid- and high-dose group were considered to be treatment-related by the study director (see Table B.6.6-47 below). However, the two confirmed misdosings in the control, the absence of signs of toxicity at 100 mg/kg bw and the absence of mortality in this dose range in the considerably high number of parallel studies shed serious doubt on a relation to treatment at this dose level. Further, various findings at gross necropsy were noted in the lungs and trachea for the 100 and 500 mg/kg/day dose groups; these findings suggest possible gavage errors, which could be responsible for some of the deaths observed at these doses and are not appropriately discussed in the report.

Table B.6.6-47: Summary of mortality in dams

Parameter	Dose Group (mg/kg bw/day)			
	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 wrong gavaging

Clinical observations

Signs of toxicity were observed at the 500 mg/kg bw/day dose group and were predominantly gastrointestinal effects, which included soft stool/liquid faeces and soft stool with mucus. Further signs of toxicity were rales, weakness, dyspnoea and ocular discharge.

Table B.6.6-48: Summary of relevant clinical signs in dams

Parameter / clinical sign	Dose Group (mg/kg bw/day)			
	0 (control)	20	100	500
Mated females	26	17	16	15
Pregnant at termination	20	13	12	6
Rales	1	0	0	3
Soft stool with mucus	0	0	0	2
Soft stool/liquid faeces	0	0	1	12
Weak	0	0	0	2
Ocular discharge	0	0	0	1
Dyspnoea	0	0	0	1

Body weight

No treatment-related and dose-related significant changes were observed in maternal body weight and body weight gain between the control, low- and mid-dose groups. In the high-dose group, initial body weight and body weights at the different time intervals were significantly lower than in the control group.

Table B.6.6-49: Summary of maternal body weight data

Parameter	Historical positive control#	Dose Group (mg/kg bw/day)			
		0 (control)	20	100	500
Number of dams pregnant at termination		20	13	12	6
Mean body weights (kg)					
Day 0		3.1	2.8	3.0	2.6*
Day 6		3.2	3.0	3.0	2.8*
Day 18		3.2	3.1	3.1	2.8
Day 28		3.3	3.3	3.3	3.0*
Day 28 (body weight – uterine weight)	2.7*	3.0	3.0	2.9	2.7
Mean body weight gain (kg)					
Day 0 – 6 (Pre-treatment)	0.2	0.1	0.2	0.0	0.1
Day 6 – 18 (Treatment)	-0.1	0.1	0.1	0.1	0.0
Day 18 – 28 (Post treatment)	0.2	0.1	0.2	0.2	0.2
Day 0 – 28 (Throughout gestation)	0.3	0.2	0.5**	0.3	0.3

Treatment with acetylsalicylic acid (ASA) at 200 mg/kg bw/day

* Significantly lower than controls by Dunnett's test $P \leq 0.05$

** Significantly higher than controls by Dunnett's test $P \leq 0.05$

Pathology**Necropsy**

Gross examination of dams at post-mortem did not identify any treatment-related effects. However, various findings were noted in the lungs and trachea for the 100 and 500 mg/kg/day dose groups which suggest possible gavage errors and issues with animal husbandry.

Observations on the ovary and uterus

A total of 20, 13, 12 and 6 pregnant females survived to termination and 134, 80, 78 and 28 fetuses were recorded for the 0 (control), 20, 100 and 500 mg/kg bw/day dose groups, respectively, and were included in the assessment. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss (complete resorptions) was recorded for

one female in the 500 mg/kg bw/day dose group; otherwise, the incidence of dams with any resorptions did not show any treatment-related differences.

There were no significant intergroup differences in the mean numbers of corpora lutea, pre-implantation and post-implantation losses and resorptions (embryonic and foetal) (see Table B.6.6-50).

Table B.6.6-50: Summary of maternal observations

Parameter	Historical positive control#	Dose group (mg/kg bw/day)			
		0 (control)	20	100	500
Mated females	12	26	17	16	15
Total number of deaths	4	2	0	4	8
Pregnant at termination	7	20	13	12	6
Mean number of corpora lutea	9	11	10	10	9
Mean number of implantations	8	8	8	9	6
Total number of embryonic resorptions (%)	6 (11)	10 (7)	11 (11)	11 (11)	9 (24)
Total number of foetal resorptions (%)	2 (4)	8 (5)	7 (7)	13 (13)	1 (3)
Total number of pre-implantation loss (%)	10 (19)	72 (48)	28 (29)	20 (20)	14 (37)
Total number of post-implantation loss (%)	8 (15)	18 (12)	18 (18)	24 (24)	10 (26)
Number of dams with any resorptions (%)	2 (29)	12 (60)	11 (85)	9 (75)	2 (33)
Dams with complete resorptions (%)	1 (14)	0 (0)	0 (0)	0 (0)	1 (17)

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw

Developmental parameters

Number and viability of foetuses

Because of the large number of maternal deaths at 500 mg/kg/day (and thus, the reduced number of total litters), the total number of foetuses was substantially less in this dose group compared to the other dose groups. However, the mean litter size, the mean numbers of abnormal, dead or live foetuses and the sex ratios of foetuses did not show any significant treatment-related differences. Glyphosate also did not cause an increase in the number of foetal deaths in utero (see Table B.6.6-51).

Foetal body weights

Although foetal body weights in the 20 and 100 mg/kg/day dose groups were reported to be significantly different from control, the weights were increased, the changes were less than 10 % of control values and no dose-response across treatment groups was evident. Thus, the foetal body weight differences observed in these two dose groups are biologically inconsequential with respect to adverse effects (see Table B.6.6-51).

Table B.6.6-51: Mean litter data at caesarean section

	Historical positive control#	Dose group (mg/kg bw/day)			
		0	20	100	500
Mated females	12	26	17	16	15
Total number of deaths	4	2	0	4	8
Pregnant at termination	7	20	13	12	6
Number of litters	6	20	13	12	5
Total number of fetuses	46	134	80	78	28
Mean litter size	8	7	6	7	6
Abnormal fetuses (%)	0 (0)	1 (1)	2 (3)	0	0
Dead fetuses (%)	0 (0)	0 (0)	1 (1)	1 (1)	0 (0)
Post-implantation loss (%)	8 (15)	18 (12)	18 (18)	24 (24)	10 (26)
Number of live fetuses	46	133	77	77	28
Mean weight of live fetuses (g \pm SD)	29 \pm 1.4	32 \pm 5.3	35 \pm 3.7*	35 \pm 2.4*	33 \pm 4.9
Sex ratio (Male : Female)	1 : 1.3	1 : 0.7	1 : 1.2	1 : 1.2	1 : 1.8

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw

SD = standard deviation

* Significantly higher than controls by Dunnett's test $P \leq 0.05$ **External, visceral and skeletal examination**

The incidence of major external malformations did not identify any treatment-related differences; further, none of the external malformations occurred in the highest dose group.

Visceral examination noted no significant treatment-related incidences of minor malformations or variants. Major visceral malformations primarily affected the heart, but occurred in single incidences and showed no dose-response (see Table B.6.6-52). The exception was dilated heart, which was reported in four fetuses of 3 litters in the 20 mg/kg bw/day dose group, 4 fetuses (3 + 1) from 2 litters of the 100 mg/kg bw/day dose group and all fetuses (4) of one litter and one fetus of another litter at the 500 mg/kg bw/day (Statistically significant $P \leq 0.05$). The terminology used to describe the heart malformations in this study is different than that typically employed in teratology research (e.g., dilated heart, seal-shaped heart). Consequently, what is meant by the description "dilated heart" is not well defined and not documented with photographs or retained tissue sections or slides. How this malformation might relate to others reported in the heart (i.e., dilated left or right ventricle, seal-shaped heart, cardiomegaly) is not clear. Further, because too few fetuses were available for examination in the high dose group, it cannot be determined whether these defects exhibited a true dose-related increase. It is important to note, however, that only 2 litters exhibited major visceral malformations in the high dose group. Additionally, these findings were found in the presence of extensive maternal toxicity, evidenced by reduced food consumption and body weight gains in the few animals that survived this dose level, clinical signs, and substantial deaths.

Major, minor and skeletal malformations did not show any clear treatment-related findings and appeared to be incidental

Table B.6.6-52: Summary of relevant external, visceral and skeletal findings (litter data)

Foetal findings	HC	Dose level (mg/kg bw/day)			
	Data [#]	0	20	100	500
No. of litters examined	6	20	13	12	5
No. of foetuses examined	46	133	79	77	28
Minor external malformations					
Percentage of small foetuses (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Major external malformations					
Percentage of foetuses with upper cleft palate (%)	0	0.8	2.5	0	0
Litter incidence (%)	0	5	15	0	0
Percentage of foetuses with forelimb arthrogryposis	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with multiple malformations	0	0.8	2.5	0	0
Litter incidence (%)	0	5	15	0	0
Percentage of foetuses with major malformations (%)	0	1.5	2.5	1.3	0
Litter incidence (%)	--	10	7.7	8.3	0
Major visceral malformations					
Percentage of foetuses with dilated heart (%)	--	0	5.1	5.2	17.9
Litter incidence (%)	--	0	23.1	16.7	40.0
Percentage of foetuses with anencephaly (%)	0	0.8	0	0	0
Litter incidence (%)	0	5.0	0	0	0
Percentage of foetuses with heart-seal shaped (%)	0	0.8	0	0	0
Litter incidence (%)	0	5.0	0	0	0
Percentage of foetuses with cardiomegaly & sealed heart (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with dilated ventricle (left) (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with dilated ventricle (right) (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Percentage of foetuses with persistent truncus arteriosus (%)	--	0.8	0	0	0
Litter incidence (%)	--	5.0	0	0	0
Percentage of foetuses with gallbladder absent (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Percentage of foetuses with liver (median) haematoma (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Minor skeletal malformations					
No. of foetuses with extra 13 th rib		0	1	2	1
Percentage of foetuses with extra 13 th rib	8.7**	0	1.3	2.6	3.6*
Litter incidence (%)	--	0	7.7	16.7	20
Major skeletal malformations					
Percentage of foetuses major malformations (%)	10.9	8.3	6.3	0*	3.6
Litter incidence (%)	50	20	23.1	0	20

[#] Historical positive control data (--: no data available)

* Significantly different from control at $p < 0.05$.

** Significantly different from control by Contingency test ($P \leq 0.05$)

Conclusion by the Notifiers

Glyphosate technical was not considered to be teratogenic in this developmental toxicity study in rabbits. The incidence of one visceral effect, dilated heart, was increased at the highest test dose and was present at lower dose levels, but there were too few foetuses present in the high dose group to corroborate a dose-response relationship. Further, foetal findings at the highest test dose were observed in the presence of extensive maternal toxicity that exceeded guideline recommendations for a high dose. The NOAEL for maternal toxicity was

100 mg/kg bw/day based on mortalities at dose levels of ≥ 100 mg/kg bw/day. Mortality and clinical signs of toxicity including reduced feed consumption and soft faeces and reduced bodyweight gain during the dosing period one incidence of complete resorptions at the 500 mg/kg bw/day dose level. The NOAEL for foetotoxicity and teratogenicity was 100 mg/kg bw/day based on occurrence of general signs of secondary toxicity (incomplete ossification and similar) at the high dose.

Comment by RMS (Re-evaluation):

The study is considered supplementary due to several weaknesses including a small number of litters for examination (low pregnancy rate at all dose levels, lethality in mid and high dose dams) and reporting deficiencies. The percentage of foetuses with 'dilated heart' was significantly increased at all dose levels. However, the absolute number of affected foetuses and litters is quite small and did not show a marked difference between the treated groups. Furthermore, the diagnosis 'dilated heart' was not defined in this study report and neither criteria used to this diagnosis nor measurements of the heart were provided.

The NOAEL for maternal toxicity is still considered 20 mg/kg bw/d, because it cannot be excluded, that the intercurrent four deaths at 100 mg/kg bw/d were substance-related.

Therefore, the previous evaluation in 2001 is confirmed: the maternal NOAEL is considered 20 mg/kg bw/d and the developmental NOAEL 100 mg/kg bw/d.

Comment by GTF on the first draft of the RAR (July 2013):

The GTF seriously questions whether the findings reported in the Suresh study were directly related to treatment. The GTF respectfully suggests that the additional safety margin for risk assessment (disregarding the 75 mg/kg/day NOAEL) as proposed by the RMS, is not necessary.

Further explanation:

Whilst an increase in mortality was observed in the Suresh study, it is uncertain if the mortalities observed were directly related to treatment, given that clinical signs of rales and dyspnoea were observed and that various findings at gross necropsy were noted in the lungs and trachea for animals in the 100 and 500 mg/kg/day dose groups. These findings suggest possible gavage errors, may well be responsible for some of the deaths observed at these doses. These findings, along with the confirmed gavage traumas in the control group call into question the technical competency of the dosing procedure in the conducting laboratory. This would likely have caused additional stress to the animals during the dosing procedure and rabbits are highly susceptible to stress induced mortality.

RMS comment (August 2013):

Not agreed. According to the study report, one gavage error was reported in the control group, but not at 100 and 500 mg/kg bw/d. Assuming technical incompetency as the main reason for lethality at mid and high dose level, one would expect the same observations at lowest dose level. Furthermore, at highest dose level 3 out of 8 animals died post-treatment, which can not be related to administration procedure, definitely.

Reference: IIA, 5.6.11/07
Report: [REDACTED] (1989)
Rabbit Teratology Study with Glyphosate Technical
[REDACTED]
Data owner: Excel
Study no.: IIT Project No. 1086
Date: 1989-11-03
not published
TOX9551960
Guidelines: OECD 414 (1981)
Deviations: no uterine weight, no maternal necropsy findings
GLP: no
Acceptability: See RMS comment

Dates of experimental work: 1989-07-03 to 1989-11-02

Materials and methods

Test material:
Identification: Glyphosate technical
Description: White amorphous powder
Lot #: 38
Purity: 95 %
Stability of test compound: Not reported
Vehicle and/
or positive control: 0.1 % gum acacia in water
Test animals:
Species: Rabbit
Strain: New Zealand White
Source: [REDACTED]
Age: 24 - 28 weeks
Sex: Females
Weight at dosing: 1.50 – 2.00 kg
Acclimation period: 6 days
Diet/Food: Pelleted rabbit feed supplied by Lipton India Ltd.,
Bangalore, India
Water: Tap water, *ad libitum*, supplied in polypropylene bottles by
Maharashtra Industrial Development Corpn., New Bombay
Housing: Individually in stainless steel cages equipped with food and
water dispensers and stainless steel grate at bottom
Environmental conditions: Temperature: 20 ± 3 °C
Humidity: 30 to 70 %
Air changes: not reported
12 hours light/dark cycle

Study design and methods:

In life dates: 1989-07-03 to 1989-11-02

Animal assignment and treatment:

In a teratogenicity study groups of 15 New Zealand White female rabbits received doses of 0, 125, 250 and 500 mg/kg bw/day test substance in 0.1 % gum acacia in water by gavage from Gestation Day 6-18 after successful mating with adult vigorous males. The day of mating was taken as the 1st day of pregnancy.

Diet preparation and analyses

For each dose level, dosing solutions were prepared in 0.1 % gum acacia in water as vehicle.

Clinical observations

A check for clinical signs of toxicity, ill-health or behavioural changes was made twice daily (before and after dosing) during the dosing period.

Body weight

Individual body weights were recorded on Days 0, 6, 12, 18, 23, and 29 (at necropsy). Changes in body weight were calculated and recorded as group maternal weight changes for the periods of Days 0-6 (pre-exposure), 6-12, 12-18, 18-23, 23-29 and 18-29 (post-exposure observation period).

Food consumption

Food consumption was recorded on Days 0, 6, 12, 18, 23, and 29 (at necropsy).

Sacrifice and pathology

Females were euthanatized by carbon dioxide asphyxiation on Day 29 of gestation and examined for any abnormalities that would affect pregnancy.

The ovaries and uteri were removed, the uteri were weighed, and the ovaries were examined for the number of corpora lutea and uteri for the number and position of implants and dead or live foetuses. Uteri from non-gravid females were placed in 10% ammonium sulfide solution for detection of early resorptions.

Developmental parameters

Each rabbit foetus was removed from the uterus and was killed by injection of pentobarbitone. All live foetuses were weighed and examined for external malformations including cleft palate and variations. All live foetuses were examined for thoracic and visceral abnormalities, and each foetus was sexed. Following visceral examination, all foetuses were eviscerated and processed for skeletal staining with Alizarin Red S. All foetuses were decapitated and heads were fixed in Bouin's solution for examination of craniofacial structures.

Statistics

Not reported.

Results and discussion

Analysis of dose formulations

The analytical purity of test substance was stated to be 95 %.

Food consumption

Mean food consumption in the low and mid dose groups was comparable to that in the control group throughout the study period. Significantly lower food consumption (~17 % lower mean

food consumption compared to control, low or mid dose group) was observed in the high dose group starting with the day of treatment throughout the rest of the observation period.

Mortality

None of the rabbits died during the study period.

Clinical observations

No toxic symptoms were observed in any of the animals during the study.

Body weight

Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, the mean maternal weight increase was lower for each of the observation periods between Days 12-29 compared to controls, but no statistical comparison was provided in the report.

Pathology

Necropsy

No abnormalities that could affect pregnancy were reported at maternal necropsy.

Observations on the ovary and uterus

Two animals of the high dose group aborted (see Table B.6.6-53).

Table B.6.6-53: Gestational parameters in rabbits treated with glyphosate

Gestational parameter	Dose level (mg/kg bw/day)			
	0	125	250	500
No. of pregnant females	15	15	15	15
No. of early deliveries	0	0	0	0
No. of abortions	0	0	0	2
No. of females with no live foetuses	0	0	0	2
No. nonpregnant at termination	2	1	1	3
No. of litters	13	14	14	12
Mean no. of corpora lutea per doe	10.0	10.1	10.3	9.8
Mean no. of total implants per litter	9.0	9.3	9.4	8.5
Mean % pre-implantation loss	21.3	14.9	14.7	13.1
Mean no. of viable implants per litter	7.3	8.0	8.0	5.2
Mean no. of non-viable implants per litter	0.07	0.13	0.27	1.4
Mean no. of early resorptions per litter	1.7	1.1	1.0	1.9
Sex ratio (% males)	44.4	49.2	49.7	50.1
Mean foetal body weight per litter	40.6	47.1	47.5	48.7

Developmental parameters

Number and viability of foetuses

The mean number of viable implants (foetuses) per litter was lower in the high dose group, and accordingly, the mean number of non-viable implants (foetuses) per litter was greater in the high dose group (see Table B.6.6-53), but no statistical comparisons were provided in the report.

Sex ratio, foetal body weights and placental weights

No differences were noted in the sex ratios, mean foetal body weights, mean number of corpora lutea per dose, mean number of total implants per litter, mean percentage of pre-implantation loss, and mean number of early resorptions between the control and the treated

groups. In the high dose group, two dams had no live foetuses due to abortions (see Table B.6.6-53). However, stastical analyses were provided in the report.

External, visceral and skeletal examination

No difference was noted in the incidences of maternal animals having foetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the incidences of external, visceral and skeletal malformations were higher than that in the control group (see Table B.6.6-54). With regard to the heart malformations, 0, 1, 1, and 2 interventricular septal defects were observed in the 0, 125, 250, and 500 mg/kg bw/day dose groups.

A similar pattern was seen in the variations observed externally, visceraally and skeletally; in the high dose group, the total number of observed variations was higher than those of the control, low or mid dose groups. The increase in malformations and variations observed in the high dose group occurred in the presence of maternal toxicity (reduced food consumption and body weight gains). Further, this was at a dose (500 mg/kg bw/day) that caused significant toxicity, including mortality, in another rabbit developmental study. However, stastical analyses were provided in the report.

Table B.6.6-54: Incidence of foetal malformations and variations in rabbits treated with glyphosate

Foetal findings	Dose level (mg/kg bw/day)			
	0	125	250	500
Malformations				
No. of litters examined	13	14	14	12
No. of foetuses examined	109	113	120	78
No of litters with malformations	3	6	10	12
% of litters with malformations	23.08	42.86	71.43	100
No. of foetuses with malformations	3	6	10	20
% of foetuses with malformations	2.75	5.31	8.33	25.64
Number of foetuses (litters) with external malformations				
Tail abnormal	1 (1)	1 (1)	2 (2)	3 (2)
Low-set ears	0 (0)	1 (1)	1 (1)	2 (1)
Total external malformations	1	2	3	3
Total external malformations (%)	0.92	1.77	2.50	3.85
Number of foetuses (litters) with visceral malformations				
Ventricular septal defect	0 (0)	1 (1)	1 (1)	2 (2)
Postcaval lung lobe absent	0 (0)	1 (1)	2 (2)	4 (3)
Kidney(s) absent	1 (1)	2 (2)	2 (2)	6 (4)
Total visceral malformations	1	4	5	12
Total visceral malformations (%)	0.92	3.54	4.17	15.38
Number of foetuses (litters) with skeletal malformations				
Rudimentary rib (no. 14)	1 (1)	0 (0)	2 (2)	5 (2)
Total skeletal malformations	1	0	2	5
Total skeletal malformations (%)	0.92	0.00	1.67	6.41
Variations				
No. of foetuses examined	109	113	120	78
Total no. of observed variations	26	30	49	93
Number of foetuses (litters) with external variations				
Tail blunt tipped	1 (1)	0 (0)	3 (2)	5 (4)
Number of foetuses (litters) with visceral variations				
Irregular rugae on palate	0 (0)	2 (1)	3 (2)	2 (2)
Lateral ventricles of cerebrum dilated	0 (0)	2 (2)	2 (2)	6 (4)
Right ventricle small than normal	1 (1)	3 (2)	3 (2)	5 (3)
Globular heart	2 (2)	0 (0)	3 (2)	5 (4)

Foetal findings	Dose level (mg/kg bw/day)			
	0	125	250	500
Incomplete separation of lung lobes	1 (1)	2 (1)	2 (1)	4 (2)
Parietal foetal atelectasis	0 (0)	1 (1)	1 (1)	1 (1)
Liver irregular shape	0 (0)	2 (1)	2 (2)	6 (4)
Kidney(s) globular shape	0 (0)	0 (0)	2 (1)	5 (3)
Number of foetuses (litters) with skeletal variations				
Cervical centra 1-3 and/or 4 bilobed	1 (1)	0 (0)	1 (1)	2 (2)
Anterior arch of the atlas poorly ossified	2 (1)	2 (1)	1 (1)	4 (2)
Anterior arch of the atlas split	0 (0)	0 (0)	2 (1)	3 (1)
Extra thoracic centrum and arch	1 (1)	3 (2)	2 (1)	5 (3)
Thoracic centrum only one ossification centre	1 (1)	0 (0)	1 (1)	3 (2)
Thoracic centra fused	2 (1)	1 (1)	1 (1)	2 (1)
Extra ribs on thoracic centra and arch 13 bilateral	1 (1)	0 (0)	3 (2)	5 (4)
Sternebra 6 poorly ossified	2 (1)	1 (1)	2 (1)	4 (2)
Sternebra(e) split	2 (1)	2 (1)	1 (1)	5 (3)
Sternebra(e) unossified	3 (2)	1 (1)	3 (2)	6 (4)
Pubis, poorly ossified	3 (2)	2 (2)	3 (1)	4 (3)
Some ossification in knee area	1 (1)	0 (0)	3 (2)	4 (3)
Skull bones poorly ossified	1 (1)	3 (2)	2 (1)	2 (2)
Frontal, hole in bone	0 (0)	1 (1)	2 (2)	2 (2)
Reduced number of caudal segments	1 (1)	2 (2)	1 (1)	3 (2)

Conclusion by the Notifiers

The oral administration of glyphosate to mated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 500 mg/kg bw/day. Therefore the NOAEL for reprotoxic and non-reprotoxic effects was considered to be 250 mg/kg bw/day. Considering the significantly reduced food consumption and gain in body weight at 500 mg/kg bw/day, the maternal NOAEL is 250 mg/kg bw/day.

Comment by RMS (Re-evaluation):

The study is considered supplementary due to serious reporting deficiencies (e.g. no individual data, no statistical analysis, no uterine weights, no results of maternal necropsy). The previous NOAEL for maternal and developmental toxicity is still considered to be 250 mg/kg bw/d based on reduced food consumption and body weight gain at 500 mg/kg bw/d in does

Developmental effects were visible as foetolethality and several malformations (external, visceral, skeletal) at high dose levels: The previous evaluation did not mention the external malformation in rabbits which are now reported in the present RAR (abnormal tails). Total number of foetuses per litter with malformations was higher in the groups receiving the mid and high dose level, but without statistical significance. However, it remains unclear, whether statistical analysis of the data had been performed at all. Ventricular septal defects were noted in 2 out of 78 foetuses in the high dose group (control incidence 0/109). The higher number of further visceral malformations at the top dose level was due to absent kidneys and postcaval lung lobes. Because no individual data are provided it is not identifiable, whether the malformations described were confined to single foetuses or if the foetuses were multiple malformed.

B.6.12 Published data (released since 2000)

A large number of studies on developmental and reproductive toxicity (DART) was published since 2000. These studies are reported and discussed below. Furthermore, also studies on

endocrine disruption (ED) have been included in this chapter because they are mainly related to developmental and reproductive toxicity.

Published studies on developmental toxicity, reproductive toxicity and an endocrine disrupting potential of glyphosate and glyphosate based formulations include *in vitro* studies, *in vivo* studies and epidemiological studies. Many studies since 2000 are specifically discussed in a comprehensive glyphosate DART review publication by Williams et al. (2012, ASB2012-12052). Further discussions of significant papers follow.

In addition, glyphosate was included on the US EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds to Tier 1 Screening. The US EPA published the criteria for inclusion on List 1 was strictly based on exposure potential, not hazard, specifically stating in the Federal Register (2009, ASB2012-12041); "This list should not be construed as a list of known or likely endocrine disruptors".

A consortium of glyphosate registrants in North America, the Joint Glyphosate Task Force, LLC (JGTF), coordinated the conduct of the glyphosate battery of Tier 1 screening assays under the EDSP and submitted these assays to the US EPA. The US EPA will evaluate the full battery of Tier 1 screening assays together using a weight of evidence approach, for glyphosate's potential to interact with the estrogen, androgen and thyroid endocrine pathways. The following below were submitted by the JGTF to the US EPA in early 2012 and are reviewed. However, the Agency has announced they will not release their Data Evaluation Records (DERs) for individual EDSP studies until a weight of evidence review has been completed for List 1 compounds.

In Vitro EDSP Glyphosate Studies submitted to the US EPA

- Androgen Receptor Binding (Rat Prostate Cytosol); OCSPP 890.1150
- Aromatase (Human Recombinant); OCSPP 890.1200
- Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC); OCSPP 890.1250
- Estrogen Receptor Transcriptional Activation (Human cell Line, HeLa-9903); OCSPP 890.1300; OECD 455
- Published OECD Validation of the Steroidogenesis Assay (Hecker et al., 2010, ASB2012-11840)

In Vivo EDSP Glyphosate Studies submitted to the US EPA

- Amphibian Metamorphosis (Frog) OCSPP 890.1100; OECD 231
- *In Vivo* Hershberger Assay (Rat); OCSPP 890.1600; OECD 441
- Female Pubertal Assay; OCSPP 890.1450; OECD None
- Male Pubertal Assay; OCSPP 890.1500
- Uterotrophic Assay (Rat); OCSPP 890.1600; OECD 440
- Fish Short-Term Reproduction Assay; OCSPP 890.1350; OECD 229

The glyphosate Tier 1 screening assay study reports are owned by the JGTF. The European Glyphosate Task Force (GTF) is negotiating to procure access rights to the battery of glyphosate EDSP Tier 1 screening study reports. Results of the Hershberger and Uterotrophic *in vivo* rat studies, now in the public domain, as are the published results of the OECD validation of the Steroidogenesis assay, in which glyphosate clearly had no impact on steroidogenesis, are discussed below.

Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia 2012, ASB2012-12016) for the Hershberger and Uterotrophic assays. No effects were noted for any potential for glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.

Bailey et al. (2013, ASB2013-3464) summarized the first results of the male and female Pubertal assay of this programme. Based on these results, glyphosate does not exhibit endocrine disruption in Male and Female Pubertal assays.

***In Vitro* Glyphosate DART/ED Publications**

Many *in vitro* research publications have characterised pesticide formulations, including glyphosate based formulations, as toxic and endocrine disrupting products. Researchers and editorial boards did in some cases not consider the fact that surfactants (which are often components of formulated pesticide products), by their physico-chemical nature, are not suitable test substances using *in vitro* cell models. Surfactants compromise the integrity of cellular membranes, including mitochondrial membranes, and thus confound endpoint measurements considered as representative of specific toxicological modes of action or pathways.

A laboratory at the University of Caen, France, has multiple recent publications of *in vitro* research with glyphosate and glyphosate based formulations (Richard et al., 2005, ASB2009-9024; Benachour et al., 2007, ASB2009-9018; Benachour and Seralini, 2009, ASB2012-11561; Gasnier et al., 2009, ASB2012-11629; Gasnier et al., 2010, ASB2012-11628; Gasnier et al., 2011, ASB2012-11630; Clair et al., 2012, ASB2012-11592; Mesnage et al., 2012, ASB2012-11900), with proposed extrapolations to an array of *in vivo* effects including potent endocrine disruption, aromatase inhibition, estrogen synthesis, placental toxicity, foetotoxicity, embryotoxicity and bioaccumulation. These publications are in some cases replicates of earlier studies, using different cell lines or primary cell cultures and in some cases the same data are reported again in a subsequent publication. Firstly, the *in vitro* synergism claims are conjecture, because no control groups of surfactant without glyphosate were tested. Secondly, the extrapolations to *in vivo* effects are unjustifiable based on both the unsuitability of surfactants in such test systems and the supraphysiological cytotoxic concentrations at which *in vitro* effects are reported. Again often overlooked by *in vitro* researchers and editorial boards, Levine et al. (2007, ASB2009-9030) presented convincing data demonstrating a lack of *in vitro* synergism for glyphosate with other formulation ingredients. Regarding Seralini's repeated claims of glyphosate induced aromatase inhibition in microsomes (Richard et al., 2005; TOX2005-1743, Benachour et al., 2007, ASB2009-9018; Gasnier et al., 2009, ASB2012-11629), the data are confounded and thus uninterpretable where surfactants are introduced to such *in vitro* systems. This is noted in the US EPA Aromatase Inhibition Test Guideline, OECD 890.1200, in which notes, "Microsomes can be denatured by detergents [surfactants]. Therefore, it is important to ensure that all glassware and other equipment used for microsome preparations be free of detergent residue."

Another *in vitro* publication claiming a specific developmental toxicity pathway has gained significant public attention. Paganelli et al. (2010, ASB2012-11986) conducted three *in vitro* assays, (i) frog embryos exposed to glyphosate formulation; (ii) frog embryos directly injected without injection blank negative controls; and (iii) fertilised chicken embryos exposed directly to a glyphosate formulation through a hole cut in the egg shell. Key issues

surrounding this research include irrelevant routes of exposure as well as excessively high and environmentally unrealistic doses.

Thongprakaisang et al., (2013, ASB2013-11991) submitted a study on the effects of pure glyphosate on estrogen receptors mediated transcriptional activity and their expressions. The following cell lines have been used: a hormone-dependent breast cancer, T47D, a stably EREC-luc construct transfected hormone-dependent breast cancer T47D-KBluc and a hormone-independent human breast cancer, MDA-MB231. Glyphosate (purity 98 %) was tested in concentrations from 10^{-12} to 10^{-6} M. Glyphosate exerted proliferative effects on human hormone-dependent cell lines but not in hormone-independent cell lines. Furthermore, an additive estrogenic effect between glyphosate and genistein, a phytoestrogen, was reported. The authors conclude that these *in vitro* results need further investigation in an animal study. It must be emphasised that no increase in mammary tumours was reported in any of the numerous long-term studies in rats or mice (see Vol. 3, B.6.5 and Vol. 1, B.2.6).

***In Vivo* Glyphosate DART/ED Publications**

Relatively few *in vivo* publications on glyphosate DART and ED exist in comparison with the list of *in vitro* publications. Some lack appropriate interpretation of basic toxicology; e.g. Daruich et al. (2001, ASB2012-11601) and Beuret et al. (2005, ASB2012-11564) noted rats treated with a glyphosate based formulation showed reduced food intake, reduced water intake and reduced body weight gains. However, the authors did not consider attributing the effects of altered enzyme concentrations to dehydration or restricted diets. Both studies are reviewed in Williams et al. (2012, ASB2012-12052).

Dallegrave et al. (2003, ASB2012-11600; 2007, ASB2012-2721) published results of two non-guidelines rat developmental toxicity studies, in which a glyphosate based formulation containing POEA was evaluated. However, reporting deficiencies and inconsistencies pose difficulties in data interpretation. These studies are discussed in detail in the Appendix on [REDACTED] (please refer to B.6.13).

Romano et al. (2010, ASB2012-12012) evaluated a glyphosate based formulation in a male pubertal-like assay in Wistar rats, reporting decreased preputial separation, reduced seminiferous epithelial height, increased luminal diameter of seminiferous tubules, and increased relative testicular and adrenal weights. Given the gravity of the reported findings in this publication, a review was undertaken by Kelce et al. (2010, ASB2012-11867). Most recently, Romano et al. (2012, ASB2012-12011) reported additional findings in male rats after supposed *in utero* and *post natal* exposures which include “behavioral changes and histological and endocrine problems in reproductive parameters and these changes are reflected by a hypersecretion of androgens and increased gonadal activity, sperm production and libido”. As in their first publication, Romano et al. (2012, ASB2012-12011) base their hypothesis on selectively discussed literature implicating glyphosate as an endocrine disruptor, predominantly with citations to research from the Seralini laboratory.

Kimmel et al. (2013, ASB2013-3462) analyzed the information from 7 unpublished developmental studies in rabbits and 6 developmental toxicity studies in rats to determine if glyphosate poses a risk for cardiovascular malformations. In summary, assessment of the reviewed data fails to support a potential risk for increased cardiovascular defects as a result of glyphosate exposure during pregnancy.

Chruscielska et al. (2000, ASB2013-9831) submitted a teratogenicity study in Wistar outbred rats. The used test guideline was not indicated. Doses of 0-750-1500-3000 mg/kg bw/day

have been administered from day 7-14 of pregnancy to 20 females per dose group. No embryotoxic and no teratogenic effects have been administered.

POEA DART Studies

Polyethoxylated alkylamine (POEA) surfactants are a class of non-ionic surfactant, containing a tertiary amine, an aliphatic group of variable carbon chain length and two separate sets of ethoxy (EO) chains of variable length. A dietary exposure assessment of POEAs was submitted by Bleeke et al. (2010, ASB2010-6123). This exposure assessment report also refers to the US EPA Alky Amine Polyalkoxylates Human Health Risk Assessment, which includes POEAs (<http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064809b983b>). Williams et al. (2012, ASB2012-12052) recently evaluated and detailed the results of DART studies with two different POEA surfactants.

Furthermore, a detailed comparison of the toxicity of [REDACTED] and glyphosate was submitted in the appendix “Toxicological evaluation of the POE-[REDACTED] surfactant (CAS no. 61791-26-2)” which is attached to this report.

Epidemiology Glyphosate DART/ED Publications

Several epidemiology studies in which glyphosate exposure was considered have evaluated the following range of reproductive outcomes; miscarriage, fecundity, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, attention-deficit disorder / attention-deficit hyperactive disorder (ADD/ADHD). 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). In evaluating ADD/ADHD, a positive association with glyphosate use was reported by Garry et al. (2002, ASB2012-11626), but cases were reported by parents with no clinical confirmation and the reported incidence rate of approximately 1 % for the study population was well below the general population incidence rate of approximately 7 %. Regarding *in utero* exposures, McQueen et al. (2012, ASB2012-11898) report very low measured dietary exposures, from 0.005 % to 2 % of the current glyphosate ADI in Europe. Given the low perfusion rate of glyphosate across the placenta (Mose et al., 2008, ASB2012-11914), human *in utero* exposures would be very limited.

Campana et al. (2010, ASB2013-10559) estimated the frequency of 27 birth defects in 7 geographical regions of Argentina. A sample of 21,844 newborn with birth defects was selected, ascertained from 855,220 births, between 1994 and 2007, in 59 hospitals. The study results suggested that frequencies of 14 of the 27 examined birth defects were higher in one or more regions. This study was discussed in some publications in relation to the use of glyphosate pesticides. However, Campana et al. (2010, ASB2013-10559) commented on secular trends, altitude above sea level, folic acid fortification and ethnic factors and further variables. It was not indicated that any of these variables was associated with an increased occurrence of any type of birth defects.

Further reviews on DART

Antoniou et al. (2012, ASB2012-15927) submitted a review article on “Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence”. According to the authors published studies “have raised concern regarding the potential for glyphosate and its commercial formulations to cause birth defects and other reproductive problems”. The “draft assessment report revealed that ... industry tests contained clear evidence of glyphosate-mediated teratogenicity and reproductive toxicity”. The EU adopted “an acceptable daily intake (ADI) for glyphosate that is unreliable and could potentially result in exposures that cause harm to humans.” The authors suggest that a “new

risk assessment should be conducted with full public transparency by scientists who are independent of industry.”

Lopez et al. (2012, ASB2013-10534) submitted a review article on “Pesticides used in South American GMO-Based Agriculture: a review on their effects on humans and animal models”. The authors discuss the results of genetic studies in agricultural regions in the province of Cordoba, Argentina, biomarkers in agricultural regions in the province of Santa Fe, Argentina and congenital malformations and genotoxicity in populations exposed to pesticides in Paraguay. According to the authors, human health in these areas was damaged by pesticides. However, a relation to glyphosate or another substance or pesticide was not evidenced. Nevertheless, based on the results of Paganelli et al. (2010, ASB2012-11986), it was concluded that glyphosate-based herbicides would be linked to an increased activity of the retinoic acid signaling pathways and this might explain the higher incidence of embryonic malformations and spontaneous abortions observed in populations exposed to pesticides.

Comparison of the active substance glyphosate and glyphosate containing formulations concerning DART and ED

For the active substance glyphosate a very comprehensive data package of guideline conform studies on developmental and reproductive toxicity is available. This data package was prepared over the last decades and updated within the last years.

In these submitted studies it was demonstrated that glyphosate is not a teratogenic substance. NOEL values for developmental toxicity and reproductive toxicity can be derived from the results of these studies. There are no relevant indications of an endocrine disrupting activity of the active substance glyphosate. Additionally, also in the further guideline conform toxicological studies (e.g. the subchronic and chronic toxicity studies) no indications of an endocrine disrupting activity of glyphosate (e.g. organ weight and histology of sexual organs, behaviour etc.) have been observed. Therefore, on basis of this comprehensive and high quality data package the active substance glyphosate is not considered to be an endocrine disruptor or a teratogenic substance.

Additionally to the studies which have been performed according to validated EU- and OECD guidelines a large number of studies has been published on DART and ED. Most of these studies use glyphosate containing preparations instead of the pure active substance glyphosate. However, some studies directly compare the toxicity of the active substance glyphosate and glyphosate containing preparations. Furthermore, studies have been performed on the toxicity of surfactants which are used in preparations together with glyphosate, especially [REDACTED]. The results of these surfactant studies can be compared with the results of the above mentioned guideline conform studies on glyphosate.

In result of these comparisons it can clearly be concluded that the toxicity of preparations and the toxicity of surfactants like [REDACTED] / polyethoxylated alkylamine is significantly higher than the toxicity of the active substance glyphosate.

A detailed comparison of the toxicity of tallowamin and glyphosate was submitted in the appendix “Toxicological evaluation of the POE-[REDACTED] surfactant (CAS no. 61791-26-2)” which is attached to this report. In this evaluation is clearly demonstrated that there is a significantly higher toxicity of the surfactant tallowamin with regard to all of the following endpoints investigated:

- acute oral toxicity
- acute dermal toxicity
- skin irritation
- eye irritation

- skin sensitization
- short term toxicity, rat
- short term toxicity, dog
- reproduction toxicity study, parental toxicity
- reproduction toxicity study, reproductive toxicity
- reproduction toxicity study, offspring toxicity
- developmental toxicity, rat, fetal effects

Walsh et al. (2000, ASB2012-12046) published research claiming that a glyphosate based formulation, but not glyphosate alone, adversely affected the steroidogenesis pathway by inhibiting progesterone production resulting in downstream reduction in mitochondrial levels of StAR protein. Subsequent research by Levine et al. (2007, ASB2009-9030) demonstrated no synergism between glyphosate and the surfactant since the cytotoxic effects were completely independent of glyphosate. Identical dose-response curves were noted for formulated product with and without the glyphosate active ingredient.

Further research addressing the steroidogenesis pathway confirmed glyphosate lacked endocrine disruption potential specific to this pathway. Quassinti et al. (2009, ASB2012-12007) evaluated effects on gonadal steroidogenesis in frog testis and ovaries on glyphosate and another active substance, noting that glyphosate unequivocally demonstrated no effect. Forgacs et al. (2012, ASB2012-11621) also tested glyphosate alone and demonstrated no effect on testosterone levels in BLTK1 murine leydig cells *in vitro*. Furthermore, the OECD multi-laboratory validation of the Steroidogenesis Assay used for Tier 1 screening of the US EPA EDSP, evaluated glyphosate and concluded no impact on steroidogenesis (Hecker et al., 2011, ASB2012-11840). Consequently, the US EPA considered reference to the OECD validation report sufficient for meeting the glyphosate Steroidogenesis Assay Test Order in the EDSP Tier 1 screening of glyphosate.

Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia, 2012, ASB2012-12016) for the Hershberger and Uterotrophic assays. No effects were noted for any potential for the active substance glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.

Richard et al. (2005, TOX2005-1743) studied effects of glyphosate and roundup on human placental cells and aromatase. Summarising their results they stated that “roundup is always more toxic than its active ingredient.”

In a further study from the same institute Benachour et al. (2007, ASB2009-9018) studied time- and dose-dependent effects of roundup on human embryonic and placental cells. They summarized that “in all instances, roundup ... is more efficient than its active ingredient, glyphosate...”. And in a further publication by Benachour and Seralini (2009, ASB2012-11561) it was stated “this work clearly confirms that the adjuvants in roundup formulations are not inert.” In a response to this publication by the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532) it was answered that surfactant effects ... are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis. In the most recent publication from the same institute, Mesnage et al. (in press, ASB2012-13917) the potential active principle for toxicity on human cells for 9 glyphosate-based formulations was studied. The authors summarized that “ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity”.

In a comprehensive analysis of the available literature in development and reproductive outcomes in humans and animals after glyphosate exposure, Williams et al. (2012, ASB2012-12052) summarized: “An evaluation of this database found no consistent effects of glyphosate exposure on reproductive health or the developing offspring. Furthermore, no plausible mechanism of action for such effects were elucidated. Although toxicity was observed in studies that used glyphosate-based formulations, the data strongly suggest that such effects were due to surfactants present in the formulations and not the direct result of glyphosate exposure.”

***In vitro* DART/ED publications**

Author(s)	Year	Study title
Walsh, L.P. McCormick, C. Martin, C. Stocco, D.M.	2000	Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environmental Health Perspectives Volume: 108 Number: 8 Pages: 769-776 ASB2012-12046

Abstract*

Recent reports demonstrate that many currently used pesticides have the capacity to disrupt reproductive function in animals. Although this reproductive dysfunction is typically characterized by alterations in serum steroid hormone levels, disruptions in spermatogenesis, and loss of fertility, the mechanisms involved in pesticide-induced infertility remain unclear. Because testicular Leydig cells play a crucial role in male reproductive function by producing testosterone, we used the mouse MA-10 Leydig tumor cell line to study the molecular events involved in pesticide-induced alterations in steroid hormone biosynthesis. We previously showed that the organochlorine insecticide lindane and the organophosphate insecticide Dimethoate directly inhibit steroidogenesis in Leydig cells by disrupting expression of the steroidogenic acute regulatory (StAR) protein. StAR protein mediates the rate-limiting and acutely regulated step in steroidogenesis, the transfer of cholesterol from the outer to the inner mitochondrial membrane where the cytochrome P450 side chain cleavage (P450_{scc}) enzyme initiates the synthesis of all steroid hormones. In the present study, we screened eight currently used pesticide formulations for their ability to inhibit steroidogenesis, concentrating on their effects on StAR expression in MA-10 cells. In addition, we determined the effects of these compounds on the levels and activities of the P450_{scc} enzyme (which converts cholesterol to pregnenolone) and the 3 β -hydroxysteroid dehydrogenase (3 β -HSD) enzyme (which converts pregnenolone to progesterone). Of the pesticides screened, only the pesticide Roundup inhibited dibutyl [cAMP-stimulated progesterone production in MA-10 cells without causing cellular toxicity. Roundup inhibited steroidogenesis by disrupting StAR protein expression, further demonstrating the susceptibility of StAR to environmental pollutants.

*Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	Non-standard test systems, but publication meets basic scientific principles. However, surfactant blend in Roundup confounds results.
Relevance of study:	Relevant with restrictions: Different effects of glyphosate alone and glyphosate formulations were observed. No conclusion can be drawn that the observed effects are result of glyphosate exposure.
Klimisch code:	2

Additional comments:

Glyphosate did not affect steroidogenesis in the test system.

Roundup formulation data was confounded by mitochondrial membrane damage, attributable to the surfactant in the tested formulation.

Roundup results were comprehensively addressed in Levine et al. (2007, ASB2009-9030):

Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have “indistinguishable” dose response curves for reductions in progesterone production in hCG stimulated MA-10 Leydig cells. Therefore the effect on progesterone levels shown by Walsh (2000, ASB2012-12046) were independent of glyphosate and attributable to the surfactant component of the formulation.

Comparable rates of progesterone inhibition for several different surfactants suggest a common mode of action for surfactants.

Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have almost identical concentration-dependent decreases in MTT activity in MA-10 cells, suggesting the surfactant alone was responsible for the observed cytotoxicity and effect on mitochondrial function.

The JC-1 assay demonstrated the decreased progesterone production in MA-10 Leydig cells was accompanied by loss of mitochondrial membrane potential. These results confirm StAR protein function and steroidogenesis require intact mitochondrial membrane potential.

StAR protein expression were not affected by treatments, indicating that perturbed mitochondrial membrane, not StAR protein inhibition, was responsible for the effects noted by Walsh et al. (2000, ASB2012-12046).

Author(s)	Year	Study title
Paganelli, A. Gnazzo, V. Acosta H. Lopez, S.L. Carrasco, A.E.	2010	Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signalling Chemical Research in Toxicology Volume: 23 Pages: 1586-1595 ASB2010-11410

Abstract*

The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosatebased herbicides (GBH) are used led us to undertake an

embryological approach to explore the effects of low doses of glyphosate in development. *Xenopus laevis* embryos were incubated with 1/5000 dilutions of a commercial GBH. The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior-posterior (A-P) axis. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed, rather than a surfactant or other component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in *Xenopus* embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of *otx2* expression and with the disruption of cephalic neural crest development. The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline study that is not sufficiently described for assessment. Inadequate positive and negative control experiments.
Relevance of study:	Not relevant: Irrelevant routes of exposure and inappropriately high doses. Test system not adequate for human risk assessment.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams et al. (2012, ASB2012-12052)

No pH adjustment for doses and thus effects may be in response to the acidic nature of glyphosate technical acid.
Inappropriate and irrelevant routes of exposure.
Data requires further substantiation before consideration in risk assessment.

Response 2 – Saltmiras et al. (2011, ASB2012-12015) letter to the Editor

Multiple high quality toxicological studies and expert review panels consistently agree glyphosate is not a teratogen or reproductive toxicant.
The authors' justification for this research is flawed, providing no valid basis, other than an opinion of an increase in the rate of birth defects in Argentina.
Direct injection of frog embryos and through chicken shells do not reflect real world exposure scenarios to either environmental species or humans.
Doses were excessively high and irrelevant for risk assessment purposes. Frog embryos were also bathed in glyphosate formulation at doses 9-15 times greater than the acute LC50 same species of frog. Calculating equivalent oral doses based on pharmacokinetics studies, such doses are 150000000 times greater than worst case human exposure monitoring data.

“.... the results from this research cannot be used in isolation to reach the conclusions expressed in the publication. Instead, the type of data in this research paper must be interpreted relative to all other available data on the specific materials under study and with balanced consideration for higher tier apical studies.”

Response 3 – Mulet (2011, ASB2012-11916) letter to the Editor

Notes the premise for this research is falsely based on an incorrectly cited local pediatric bulletin from Paraguay.

“.... this article refers to a study in a single hospital in Paraguay showing a correlation between pesticide use (not herbicides as mentioned by Paganelli et al., ASB2010-11410) and birth malformations. In the cited study (Benitez et al., ASB2012-11563), the authors state that the results are preliminary and must be confirmed. Is important to remark that the Benitez et al. study does not include any mention to glyphosate, so does not account for what the authors are stating in the introduction.....This journal is also wrongly cited in the discussion referring to increased malformations due to herbicides, which is not the result of the study.”

Response 4 – comments from BVL (2010, ASB2012-11579)

Highly artificial experimental conditions.

Inappropriate models to replace validated mammalian reproductive and developmental toxicity testing methods for use in human health risk assessment.

Inappropriate routes of exposure.

Lack of corroborative evidence in humans.

“In spite of long-lasting use of glyphosate-based herbicides worldwide, no evidence of teratogenicity in humans has been obtained so far.”

Response 5– comments from European Commission Standing Committee on the Food Chain and Animal Health (2011, ASB2012-11615)

The EU commission supports the German Authorities position, “that that there is a comprehensive and reliable toxicological database for glyphosate and the effects observed have not been revealed in mammalian studies, nor evidenced epidemiologically in humans.”

“.... the Commission does not consider there is currently a solid basis to ban or impose specific restrictions on the use of glyphosate in the EU.”

Response 6– Palma, G. (2010, ASB2012-11989) letter to the Editor

The author of the letter claims that the study by Paganelli et al., 2010 (ASB2010-11410), described effects of glyphosate only at unrealistic high concentrations or via unrealistic routes of exposure. The data are thought to be inconsistent with the literature, and therefore not suitable or relevant for the risk assessment for humans and wildlife. Furthermore the author asserts that findings do not support the extrapolation to human health as stated in the publication.

Author(s)	Year	Study title
Richard, S. Moslemi, S. Sipahutar, H. Benachour, N. Seralini, G.E.	2005	Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives Volume: 113 Pages: 716-720 TOX2005-1743

Abstract*

Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems, but its mechanism of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Study design is insufficient for risk assessment of real exposure concentrations. Methodological deficiencies (no controls were included). Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytotoxic membrane disruption potential of surfactants are well known for *in vitro* test systems. EPA Test Guideline OCSPP 890.1200 specifically notes that microsomes are denatured by detergents (i.e. surfactants) and that all glassware should be thoroughly rinsed.

Relevance of study:

Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.

Klimisch code:

3

Additional comments:

Response 1 – summarized from Williams et al. (2012, ASB2012-12052)

Glyphosate at non-cytotoxic concentrations in this test system was demonstrated to have no effects on aromatase activity.

Likewise, did not affect mRNA levels after 18 hours treatment at $\leq 0.1\%$ glyphosate.

Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.

The *in vitro* test system is non-validated

Physiologically irrelevant concentrations tested.

Testing surfactant-like substances in such systems is now recognized to be not valid.

Response 2 – summarized from the French Ministry of Agriculture and Fish, Committee for Study of Toxicity (2005, ASB2009-9025)

Major methodological gaps.

JEG3 cells, a choriocarcinoma human cell line (average of 70 chromosomes vs 46 in normal human cells).

Concentrations of Roundup used in the various experiments considered to be extremely high. In consideration of limiting factors (oral absorption, 30 %; skin absorption, 0.3 %; rapid elimination kinetics), such levels would involve considerable human exposure, or several dozen liters of Roundup diluted at 2 %.

concentrations of Roundup that trigger an effect on aromatase (0.5 % - 2 %) are at least 1000 times more effective than those of known aromatase inhibitors, such asazole derivatives. Study design does not make it possible to show the influence of the adjuvants, nor synergism of adjuvants and glyphosate.

Multiple non-specific effects of surfactant agents on a broad range of cellular targets not discussed.

No comparison with comparable surfactant agents intended for household use.

Multiple instances of bias in its arguments and its interpretation of the data.

The authors over-interpret their results in the area of potential health consequences for humans (unsuitable references, non-sustained in vitro-in vivo extrapolation, etc.).

Author(s)	Year	Study title
Benachour, N. Sipahutar, H. Moslerni, S. Gasnier, C. Travert, C. Seralini, G. E.	2007	Time- and dose-dependent effects of roundup on human embryonic and placental cells. Archives of Environmental Contamination and Toxicology Volume: 53 Pages: 126-133 ASB2009-9018

Abstract*

Roundup® is the major herbicide used worldwide, in particular on genetically modified plants that have been designed to tolerate it. We have tested the toxicity and endocrine disruption potential of Roundup (Bioforce®) on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. The cell lines have proven to be suitable to estimate hormonal activity and toxicity of pollutants. The median lethal dose (LD₅₀) of Roundup with embryonic cells is 0.3 % within 1 h in serum-free medium, and it decreases to reach 0.06 % (containing among other compounds 1.27 mM glyphosate) after 72 h in the presence of serum. In these conditions, the embryonic cells appear to be 2-4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1 -2 %, i.e., with 21-42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup. We demonstrated that serum-free cultures, even on a short-term basis (1 h), reveal the xenobiotic impacts that are visible 1-2 days later in serum. We also document at lower non-overtly toxic doses, from 0.01 % (with 210 µM glyphosate) in 24 h, that Roundup is an aromatase disruptor. The direct inhibition is temperature-dependent and is confirmed in different tissues and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). Furthermore, glyphosate acts directly as a partial inactivator on microsomal aromatase, independently of its acidity, and in a dose-dependent manner. The cytotoxic, and potentially endocrine-disrupting effects of Roundup are thus amplified with time. Taken together, these data suggest that Roundup exposure may affect human reproduction and fetal development in case of contamination. Chemical mixtures in formulations appear to be underestimated regarding their toxic or hormonal impact.

Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study report has several reporting deficiencies in the methods section (e.g. test conditions for the pH- and temperature dependent assay not reported). There is no information on the suitability of the used HEK 293 cell line for assessment of hormonal activity. Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytotoxic membrane disruption potential of surfactants are well known for <i>in vitro</i> test systems.
Relevance of study:	Not relevant: Excessive doses exceed typical <i>in vitro</i> limit doses. <i>In vitro</i> test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:

Glyphosate at and above relevant concentrations for this test system was demonstrated to have no effects on aromatase activity.

Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.

Comparable research to Richard et al (2005, TOX2005-1743), but with an additional cell line, HEK 293, derived from aborted human embryo kidneys, transformed by inserting adenovirus DNA.

Excessively high doses tested, not environmentally relevant for human health or environmental risk assessment.

Aromatase production within the steroidogenesis pathway. Therefore, aromatase inhibition would be detected in the steroidogenesis assay. The OECD multi-laboratory validation of the steroidogenesis assay evaluated glyphosate, demonstrating no impact on the steroidogenesis pathway (Hecker et al., 2011, ASB2012-11840).

Response – summarized from Williams et al. (2012, ASB2012-12052)

pH of test system not adjusted to physiologically appropriate levels;

Negative controls were not pH adjusted to appropriate levels.

Confounding surfactant effects due to cell membrane damage render data generated with formulated products in this test system null.

Author(s)	Year	Study title
Benachour N. Seralini G. E.	2009	Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chemical Research in toxicology Volume: 22, Pages: 97-105 ASB2012-11561

Abstract*

We have evaluated the toxicity of four glyphosate (G)-based herbicides in Roundup formulations, from 10(5) times dilutions, on three different human cell types. This dilution level is far below agricultural recommendations and corresponds to low levels of residues in food or feed. The formulations have been compared to G alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA. HUVEC primary neonate umbilical cord vein cells have been tested with 293 embryonic kidney and JEG3 placental cell lines. All R formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage. They also induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells. G provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants. AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. This should be discussed when analyzing the *in vivo* toxic actions of R. This work clearly confirms that the adjuvants in Roundup formulations are not inert. Moreover, the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from formulation-treated crops.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytotoxic membrane disruption potential of surfactants are well known for <i>in vitro</i> test systems. No positive controls were included.
Relevance of study:	Not relevant (Excessive doses exceed typical <i>in vitro</i> limit doses. <i>In vitro</i> test system is inappropriate with surfactants)
Klimisch code:	3

Additional comments:

Response – summarized from the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532)

Cell lines used present characteristics which may be at the source of a significant bias in the interpretation of the results.

Experiments were conducted with 24 hours exposure in a medium without serum, which could lead to disturbance of the physiological state of the cells.

The glyphosate used in the study is glyphosate acid, whereas in the preparations tested it is in the form of an isopropylamine salt. No precise information is given about the pH of test concentrations except the highest dose.

No mention of any positive evidence for the apoptosis test.

Cytotoxicity and induction of apoptosis may be due to pH and/or variations in osmotic pressure on cell survival at the high doses tested.

Surfactant (tensoactive) effects and increased osmolality are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis.

Conclusions are based on unvalidated, non-representative cell models (in particular tumour or transformed cell lines) directly exposed to extremely high product concentrations in culture conditions which do not observe normal cell physiological conditions.

No new information is presented on mechanism of action of glyphosate and preparations containing glyphosate.

The authors over-interpret their results with regard to potential health consequences for humans, based in particular on an unsupported *in vitro*–*in vivo* extrapolation.

The cytotoxic effects of glyphosate, its metabolite AMPA, the tensioactive POAE and other glyphosate-based preparations proposed by Benachour and Seralini do not add any pertinent new facts which call into question the conclusions of the European assessment of glyphosate or those of the national assessment of the preparations.

Author(s)	Year	Study title
Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M. C., Seralini, G. E	2009	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology Volume: 262 Number: 3, Pages: 184-191 ASB2012-11629

Abstract*

Glyphosate-based herbicides are the most widely used across the world; they are commercialised in different formulations. Their residues are frequent pollutants in the environment. In addition, these herbicides are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. Up to 400 ppm of their residues are accepted in some feed. We exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity to four different formulations and to glyphosate, which is usually tested alone in chronic *in vivo* regulatory studies. We measured cytotoxicity with three assays (Alamar Blue, MTT, ToxiLight), plus genotoxicity (comet assay), anti-estrogenic (on ER α , ER β) and anti-androgenic effects (on AR) using gene reporter tests. We also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters were disrupted at sub-agricultural doses with all formulations within 24h. These effects were more dependent on the formulation than on the glyphosate concentration. First, we observed a human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. A real cell impact of glyphosate-based herbicides residues in food, feed or in the environment has thus to be considered, and their classifications as carcinogens/mutagens/reprotoxics is discussed.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Due to reporting deficiencies (e.g. correlation between

concentration used in toxicity tests and concentrations used in comet assay) assessment of results difficult. Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytotoxic membrane disruption potential of surfactants are well known for *in vitro* test systems.

Relevance of study: Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.

Klimisch code: 3

Additional comments:

Response 1 – summarized from Williams et al. (2012, ASB2012-12052)

Glyphosate demonstrated no significant anti-estrogenic potential. Glyphosate demonstrated some anti-androgenic potential at lower concentrations, but not as doses increased and therefore results are considered unrelated to treatment. Four glyphosate based formulations demonstrated both estrogenic and androgenic activity. Results are confounded due to surfactants within the formulated products tested, which affect cell membrane integrity and produces false findings.

Response 2 – summarized from BfR Review (2009, ASB2012-11565)

Numerous methodological flaws are noted.
 Test substance(s) not characterized
 Source of materials for cell culture not provided.
 Dosing concentrations not well described.
 Serum free media only appropriate for short term (3-4 hour) *in vitro* exposures.
 pH control of dilutions not clear.
 Osmolality of test solutions not reported.
 Electrophoresis parameters insufficiently or inaccurately reported.
 Numerous reporting deficiencies are noted.
 Influence of serum-free cell culturing on endpoints can not be determined
 Incomplete data reporting, including β -galactosidase activity, cytotoxicity for select assays.
 Positive control data not reported.
 Confusion between maximum residue levels versus systemic concentrations in humans.

Author(s)	Year	Study title
Clair, E., Mesnage, R., Travert, C., Seralini, G.E.	2012	A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells <i>in vitro</i> , and testosterone decrease at lower levels. Toxicology in Vitro Volume: 26, Number: 2, Pages: 269-279 ASB2012-1628

Abstract*

The major herbicide used worldwide, Roundup, is a glyphosate-based pesticide with adjuvants. Glyphosate, its active ingredient in plants and its main metabolite (AMPA) are among the first contaminants of surface waters. Roundup is being used increasingly in

particular on genetically modified plants grown for food and feed that contain its residues. Here we tested glyphosate and its formulation on mature rat fresh testicular cells from 1 to 10000 ppm, thus from the range in some human urine and in environment to agricultural levels. We show that from 1 to 48 h of Roundup exposure Leydig cells are damaged. Within 24–48 h this formulation is also toxic on the other cells, mainly by necrosis, by contrast to glyphosate alone which is essentially toxic on Sertoli cells. Later, it also induces apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures. At lower non toxic concentrations of Roundup and glyphosate (1 ppm), the main endocrine disruption is a testosterone decrease by 35%. The pesticide has thus an endocrine impact at very low environmental doses, but only a high contamination appears to provoke an acute rat testicular toxicity. This does not anticipate the chronic toxicity which is insufficiently tested and only with glyphosate in regulatory tests.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline <i>in vitro</i> test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified).
Relevance of study:	Not relevant (Due to reliability. In addition, <i>in vitro</i> data do not reflect real <i>in vivo</i> exposure situations, and therefore not relevant for human risk assessment purposes.)
Klimisch code:	3

Additional comments:

In vitro test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified). The concentrations used in these experiments are not relevant to human exposures to glyphosate and the experimental system used is not relevant to whole animal outcomes. Importantly, the alleged impacts on endocrine function have not been observed in animal studies of glyphosate or other components of glyphosate formulations at relevant concentrations. Authors state that the lowest concentration of glyphosate tested was 50 ppm, several orders of magnitude higher than an anticipated human intake (based on pharmacokinetics described in Anadon et al., 2009, ASB2012-11542) following worst case dietary exposure at the ADI.

Author(s)	Year	Study title
Hokanson, R. Fudge, R. Chowdhary, R. Busbee, D.	2007	Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Human & Experimental Toxicology Volume: 26, Pages: 747-752, ASB2012-11846

Abstract*

Gene expression is altered in mammalian cells (MCF-7 cells), by exposure to a variety of chemicals that mimic steroid hormones or interact with endocrine receptors or their co-factors. Among those populations chronically exposed to these endocrine disruptive

chemicals are persons, and their families, who are employed in agriculture or horticulture, or who use agricultural/horticultural chemicals. Among the chemicals most commonly used both commercially and in the home, is the herbicide glyphosate. Although glyphosate is commonly considered to be relatively non-toxic, we utilized *in vitro* DNA microarray analysis of this chemical to evaluate its capacity to alter the expression of a variety of genes in human cells. We selected a group of genes, determined by DNA microarray analysis to be dysregulated, and used quantitative real-time PCR to corroborate their altered states of expression. We discussed the reported function of those genes, with emphasis on altered physiological states that are capable of initiating adverse health effects that might be anticipated if gene expression were significantly altered in either adults or embryos exposed *in utero*.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Not acceptable <i>in vitro</i> methods for test mixtures containing surfactant. Well documented study publication, but surfactants are inappropriate test substance in cell lines.
Relevance of study:	Not relevant Temporal altered gene expression is not a biomarker for toxicity, but rather, may be within the range of normal biological responses of homeostasis. <i>In vitro</i> cytotoxicity of surfactants, however, is a significant confounder in data interpretation. Data do not reflect real <i>in vivo</i> exposure situations, and therefore not relevant for human risk assessment purposes.
Klimisch code:	3

Additional comments:

In vitro cytotoxicity of surfactants is a significant confounder in data interpretation. Relevance of altered gene expression in a cell line derived from a breast cancer should not be extrapolated to reflect human health endpoints. Altered gene expression should not be confused with adverse health outcomes. Rather altered gene expression may equally be considered a biological response within the range of normal homeostasis.

In vivo DART/ED publications

Author(s)	Year	Study title
Yousef, M.F., Salem, M.H., Ibrahim, H.Z., Helm, S., Seehy, M.A., Bertheussen, K.	1995	Toxic Effects of Carbofuran and Glyphosate on Semen Characteristics in Rabbits. Journal of Environmental Science and Health. Part B. Volume: 30, Number: 4, Pages: 513-534 ASB2012-12058

Abstract*

The present study was undertaken to investigate the effect of chronic treatment with two sublethal doses of Carbofuran (carbamate insecticide) and Glyphosate (organophosphorus herbicide) on body weight and semen characteristics in mature male New Zealand white rabbits. Pesticide treatment resulted in a decline in body weight, libido, ejaculate volume, sperm concentration, semen initial fructose and semen osmolality. This was accompanied with increases in the abnormal and dead sperm and semen methylene blue reduction time. The hazardous effect of these pesticides on semen quality continued during the recovery period, and was dose-dependent. These effects on sperm quality may be due to the direct cytotoxic effects of these pesticides on spermatogenesis and/or indirectly via hypothalamic-pituitary-testis axis which control the reproductive efficiency.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Non-GLP, non-guideline study with major reporting deficiencies. Dose-levels poorly defined as 1/10 and 1/100 LD₅₀. Purity of the test substances, source of animals, environmental conditions, mortality and clinical signs not reported. No testis and epididymis weights were determined or reported and no histopathological examination conducted. In addition, stability and homogeneity assessment of test substance preparations were not done or not reported. Rabbits have low body weights at study start, suggesting impaired health status.

Relevance of study:

Not relevant (Due to low confidence in study conduct and the inadequacy of reporting.)

Klimisch code:

3

Additional comments:

Response – summarized from Williams et al. (2000, ASB2012-12053)

Numerous serious deficiencies in the design, conduct, and reporting of this study which make the results uninterpretable.

Only four rabbits per treatment group were used, and therefore statistics are questionable.

Rabbits appeared to be small for their age; at study start (32 weeks) tested animals had 16-25 % lower body weight than historical weights for commercially bred animals of the same age and strain.

Low body weights as study start suggest compromised health status of the animals at initiation.

Dose levels were not quantified.

Purity of glyphosate and composition of the glyphosate formulation were not reported.

Inadequate description of test material administration.

Improper semen collection technique reported.

Report is unclear whether control animal sham handling was undertaken, a critical factor in stress related outcomes in this species.

Food consumption of test and control groups not adequately reported.

Variability not adequately reported for endpoint measurements in test and control groups, preventing statistical analysis to support the author's conclusions.

Dose-responses not observed, despite the wide dose spread.
Sperm concentrations of all groups within normal ranges for this strain of rabbit.
No meaningful conclusions can be drawn from this publication.

Author(s)	Year	Study title
Daruich, J. Zirulnik, F. Gimenez, M. S.	2001	Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses Environmental Research Volume: 85 Pages: 226-231 ASB2012-11601

Abstract*

To prevent health risk from environmental chemicals, particularly for progeny, we have studied the effects of the herbicide glyphosate on several enzymes of pregnant rats. Glyphosate is an organo-phosphorated nonselective agrochemical widely used in many countries including Argentina and acts after the sprout in a systemic way. We have studied three cytosolic enzymes: isocitrate dehydrogenase-NADP dependent, glucose-6-phosphate dehydrogenase, and malic dehydrogenase in liver, heart and brain of pregnant Wistar rats. The treatment was administered during the 21 days of pregnancy, with 1 week as an acclimation period. The results suggest that maternal exposure to agrochemicals during pregnancy induces a variety of functional abnormalities in the specific activity of the enzymes in the studied organs of the pregnant rats and their fetuses.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable
Comment: Basic data given, however, the study is performed with methodological and reporting deficiencies (unknown exposure levels, only cytosolic enzymes measured, inappropriate controls, lack of consistent dose-response data).
Relevance of study: Not relevant (Due to reliability. In addition, study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate).
Klimisch code: 3

Additional comments:

The study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate. Test substance administration is poorly described, but rough calculations on approximate surfactant intake show excessively high and unrealistic exposures when compared to DART systemic parental and reproductive/developmental NOAEL values for POEA formulation surfactants.

Response summarized from Williams et al. (2012, ASB2012-12052)

Test substance and doses not adequately described.
Inappropriate control groups.

Results suggest that the effect of treatment on body and organ weights may be due to reduced food and water intakes.

A consistent effect of treatment was not observed and dose-response relationships were generally lacking

The information gathered may be misleading because the enzymes monitored are found in both the cytosol and mitochondria.

Food restriction affects the activity of many enzymes, including those examined in this study.

Author(s)	Year	Study title
Romano, R.M. Romano, M.A. Bernardi, M.M. Furtado, P.V. Oliveira, C.A.	2010	Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Archives of Toxicology Volume: 84, Pages: 309-317 ASB2012-12012

Abstract*

Glyphosate is a herbicide widely used to kill weeds both in agricultural and non-agricultural landscapes. Its reproductive toxicity is related to the inhibition of a StAR protein and an aromatase enzyme, which causes an in vitro reduction in testosterone and estradiol synthesis. Studies in vivo about this herbicide effects in prepubertal Wistar rats reproductive development were not performed at this moment. Evaluations included the progression of puberty, body development, the hormonal production of testosterone, estradiol and corticosterone, and the morphology of the testis. Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in seminiferous tubules' morphology, decreased significantly the epithelium height ($P < 0.001$; control = $85.8 \pm 2.8 \mu\text{m}$; 5 mg/kg = $71.9 \pm 5.3 \mu\text{m}$; 50 mg/kg = $69.1 \pm 1.7 \mu\text{m}$; 250 mg/kg = $65.2 \pm 1.3 \mu\text{m}$) and increased the luminal diameter ($P < 0.01$; control = $94.0 \pm 5.7 \mu\text{m}$; 5 mg/kg = $116.6 \pm 6.6 \mu\text{m}$; 50 mg/kg = $114.3 \pm 3.1 \mu\text{m}$; 250 mg/kg = $130.3 \pm 4.8 \mu\text{m}$); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups ($P < 0.001$; control = $154.5 \pm 12.9 \text{ ng/dL}$; 5 mg/kg = $108.6 \pm 19.6 \text{ ng/dL}$; 50 mg/kg = $84.5 \pm 12.2 \text{ ng/dL}$; 250 mg/kg = $76.9 \pm 14.2 \text{ ng/dL}$). These results suggest that commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disturbances in the reproductive development of rats when the exposure was performed during the puberty period.

Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Study with methodological and reporting deficiencies or conflicting findings (e.g., increased relative testicular weights, but decreased testosterone measurements).

Relevance of study:

Relevant study type for investigating male reproductive endpoints, but questionable relevance of this specific study based on low reliability of data and interpretation. Not relevant for glyphosate (test material was a formulated product, not glyphosate).

Klimisch code:

3

Additional comments:

Test material was a formulated product, not glyphosate. The authors failed to measure many of the key parameters in the validated pubertal male assay protocol and hence generated data that were internally inconsistent or incomplete.

Author(s)	Year	Study title
Romano, M.A. Romano, R.M. Santos, L.D. Wisniewski, P. Campos, D.A. de Souza, P.B. Viau, P. Bernardi, M.M. Nunes, M.T. de Oliveira, C.A.	2012	Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression Archives of Toxicology Volume: 86, Number: 4, Pages: 663-673 ASB2012-12011

Abstract*

Sexual differentiation in the brain takes place from late gestation to the early postnatal days. This is dependent on the conversion of circulating testosterone into estradiol by the enzyme aromatase. The glyphosate was shown to alter aromatase activity and decrease serum testosterone concentrations. Thus, the aim of this study was to investigate the effect of gestational maternal glyphosate exposure (50 mg/kg, NOAEL for reproductive toxicity) on the reproductive development of male offspring. Sixty-day-old male rat offspring were evaluated for sexual behavior and partner preference; serum testosterone concentrations, estradiol, FSH and LH; the mRNA and protein content of LH and FSH; sperm production and the morphology of the seminiferous epithelium; and the weight of the testes, epididymis and seminal vesicles. The growth, the weight and age at puberty of the animals were also recorded to evaluate the effect of the treatment. The most important findings were increases in sexual partner preference scores and the latency time to the first mount; testosterone and estradiol serum concentrations; the mRNA expression and protein content in the pituitary gland and the serum concentration of LH; sperm production and reserves; and the height of the germinal epithelium of seminiferous tubules. We also observed an early onset of puberty but no effect on the body growth in these animals. These results suggest that maternal exposure to glyphosate disturbed the masculinization process and promoted behavioral changes and histological and endocrine problems in reproductive parameters. These changes associated with the hypersecretion of androgens increased gonadal activity and sperm production.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Non-guideline, non-GLP study meeting scientific principles. Unusual and short dosing regiment commencing towards the end of pregnancy (GD18, rather than GD6 as per OECD Test Guidelines 414) through post natal day 5. *In vivo* study with reporting

deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). A number of atypical endpoints evaluated.

Relevance of study: Not relevant (due to questionable dosing regimen and atypical array of endpoints measured).

Klimisch code: 3

Additional comments:

Study with some reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). Dosing was limited to dams, starting on gestation day 18, well after organogenesis, through post natal day 5. No controls for litter effects appear to be reported, confounding interpretation of results. With the very short window of maternal exposure, biological plausibility of any test substance related effects in the mature offspring is questionable. However, the normal variability of some unusual or atypical endpoint measurements, such as “sexual partner preference” along with mRNA and protein expression, is not known. Of particular concern, however, are differences in critical endpoints for control animals reported in Romano et al. (2010, ASB2012-12012) compared to Romano et al. (2012, ASB2012-12011); these include increased day of preputional separation (PPS) of control male rate (37 days in 2010; 47 days in 2012), body weight at day of PPS (146 grams in 2010; 245 grams in 2012), serum testosterone concentrations (155 ng/dL in 2010; 63 ng/dL in 2012), estradiol concentrations (32 pg/mL in 2010; 1.4 pg/mL in 2012), subular diameter (266 µm in 2010; 479 µm in 2012), epithelial height (86 µm in 2010; 92 µm in 2012) and luminal height (94 µm in 2010; 257 µm in 2012). Therefore, results are difficult to interpret, particularly for relevance to human health risk assessment.

Epidemiology DART/ED Publications

Author(s)	Year	Study title
Arbuckle, T. E. Lin, Z. Mery, L. S.	2001	An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population Environmental Health Perspectives Volume: 109 Pages: 851-857 ASB2012-11545

Abstract*

The toxicity of pesticides on human reproduction is largely unknown—particularly how mixtures of pesticide products might affect fetal toxicity. The Ontario Farm Family Health Study collected data by questionnaire on the identity and timing of pesticide use on the farm, lifestyle factors, and a complete reproductive history from the farm operator and eligible couples living on the farm. A total of 2,110 women provided information on 3,936 pregnancies, including 395 spontaneous abortions. To explore critical windows of exposure and target sites for toxicity, we examined exposures separately for preconception (3 months

before and up to month of conception) and postconception (first trimester) windows and for early (< 12 weeks) and late (12–19 weeks) spontaneous abortions. We observed moderate increases in risk of early abortions for preconception exposures to phenoxy acetic acid herbicides [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1–2.1], triazines (OR = 1.4; 95% CI, 1.0–2.0), and any herbicide (OR = 1.4; 95% CI, 1.1–1.9). For late abortions, preconception exposure to glyphosate (OR = 1.7; 95% CI, 1.0–2.9), thiocarbamates (OR = 1.8; 95% CI, 1.1–3.0), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0–2.4) was associated with elevated risks. Postconception exposures were generally associated with late spontaneous abortions. Older maternal age (> 34 years of age) was the strongest risk factor for spontaneous abortions, and we observed several interactions between pesticides in the older age group using Classification and Regression Tree analysis. This study shows that timing of exposure and restricting analyses to more homogeneous endpoints are important in characterizing the reproductive toxicity of pesticides.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide.
Relevance of study:	Not relevant (Study design is not suitable for assessment of glyphosate exposure).
Klimisch code:	3

Additional comments:

Pre-conception glyphosate exposure odds ratio for spontaneous abortion is considered of borderline significance (OR = 1.4). Post-conception glyphosate exposure was not associated with spontaneous abortion (OR = 1.1). Authors note multiple limitations of the study relating to exposure, likely misclassification of pesticides and correct assignment of exposure window to pre- or/and post-conception

OFFHS information gathering methodology has high potential recall bias. Blair and Zahm (1993, ASB2012-11567) report 60 % accuracy when comparing self reported pesticide usage with purchasing records.

OFFHS relied exclusively on maternal self-reports of adverse pregnancy outcomes, not all of which were confirmed via medical or other records.

Three highly relevant confounding factors were not considered in the OFFHS questionnaire: history of previous spontaneous abortion(s), maternal age and smoking.

Response summarized from Williams et al. (2012, ASB2012-12052)

395 spontaneous abortions were reported out of 3936 pregnancies; rate of spontaneous aborting in Arbuckle et al. (2001, ASB2012-11545) was 10 %.

The baseline rate of spontaneous abortions in the general populations is much higher, ranging from 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).

Substantial exposure misclassification may have occurred (pre- versus post-conception) due to likely author extrapolation of exposure data.

Strong confounding variables are not apparent in previous data analyses published by the authors of the OFFHS, and therefore odds ratios are crude.

Published results fail to demonstrate a significant association of glyphosate exposure spontaneous abortion risk and therefore must be considered cautiously.

Author(s)	Year	Study title
Savitz, D.A. Arbuckle, T. Kaczor, D. Curtis, K.M.	1997	Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology Volume: 146, Number: 12, Pages: 1025-1036 ASB2012-12022

Abstract*

Potential health effects of agricultural pesticide use include reproductive outcomes. For the Ontario Farm Family Health Study, the authors sampled Ontario farms from the 1986 Canadian Census of Agriculture, identified farm couples, and obtained questionnaire data concerning farm activities, reproductive health experience, and chemical applications. Male farm activities in the period from 3 months before conception through the month of conception were evaluated in relation to miscarriage, preterm delivery, and small-for-gestational-age births. Among the 1,898 couples with complete data (64 % response), 3,984 eligible pregnancies were identified. Miscarriage was not associated with chemical activities overall but was increased in combination with reported use of thiocarbamates, carbaryl, and unclassified pesticides on the farm. Preterm delivery was also not strongly associated with farm chemical activities overall, except for mixing or applying yard herbicides (odds ratio = 2.1, 95 % confidence interval 1.0-4.4). Combinations of activities with a variety of chemicals (atrazine, glyphosate, organophosphates, 4-[2,4-dichlorophenoxy] butyric acid, and insecticides) generated odds ratios of two or greater. No associations were found between farm chemicals and small-for-gestational-age births or altered sex ratio. Based on these data, despite limitations in exposure assessment, the authors encourage continued evaluation of male exposures, particularly in relation to miscarriage and preterm delivery.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not Reliable

Comment:

No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide. Due to study design and evaluation methods, study results are not reliable.

Relevance of study:

Not Relevant (Study design is not suitable for assessment of glyphosate exposure).

Klimisch code:

3

Additional comments:

Glyphosate is one of many pesticides mentioned in three epidemiological reports that examine possible links between on-farm pesticide use and reproductive outcomes. All three reports - Savitz *et al.* (1997, ASB2012-12022), Curtis *et al.* (1999, cited in ASB2012-11545) and Arbuckle *et al.* (2001, ASB2012-11545) - use data from the Ontario Farm Family Health Study (OFFHS) (Arbuckle 1994, cited in ASB2012-11545). Savitz *et al.* (1997, ASB2012-12022) investigated associations between reported pesticide use by males and pregnancy outcomes, specifically: miscarriage, pre-term delivery and small-for-gestational-age birth.

Curtis *et al.* (1999, cited in ASB2012-11545) studied whether reported pesticide use by males or females was associated with delayed pregnancy, while Arbuckle *et al.* (2001, ASB2012-11545) looked for associations between reported pesticide use and spontaneous abortion.

In the study by Savitz *et al.* (1997, ASB2012-12022), a number of specific pesticides had weak statistical associations with miscarriages and pre-term deliveries, but pesticides tended not to be associated with small for gestational age births. There were no statistically significant findings for glyphosate. In the study by Curtis *et al.* (1999, cited in ASB2012-11545), for farms on which glyphosate was used, there was no significant association for women being engaged in pesticide activities. For men, glyphosate use was associated with a slight, but statistically significant, decrease in time to pregnancy. The authors dismissed this finding, which was contrary to their hypothesis that pesticide exposure delayed pregnancy, as probably due to uncontrolled factors or chance. Arbuckle *et al.* (2001, ASB2012-11545) found that reported preconception use of phenoxyacetic acids, triazines, glyphosate, and thiocarbamates were weakly, but statistically significantly, associated with spontaneous abortions. Post conception reported use was not associated with increased risk. The authors characterized the associations between pesticides and spontaneous abortions as "hypothesis generating" pending confirmation from other epidemiologic studies.

These studies are not convincing evidence of a relationship between glyphosate exposure and adverse pregnancy outcomes for a number of reasons:

There was no actual exposure data *per se* in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. For example, according to a study by the National Cancer Institute, self-reports of pesticide usage were found to be only 60 percent accurate when compared with purchasing records (Blair & Zahm 1993, ASB2012-11567). Further increasing the potential for inaccuracy is the fact that study subjects were only asked about pesticide use for the 5 years before the OFFS survey. These responses were assumed to be applicable to the entire farming careers of study subjects, an assumption inconsistent with changes in agricultural practice. Lastly, basing exposure estimation on questionnaire responses has the potential to be influenced by what epidemiologists call "recall bias." This refers to the likelihood that families that experienced an adverse reproductive outcome are more likely to remember use of certain pesticides than families that had only normal births.

The most widely used pesticides, like atrazine, glyphosate, and 2,4-D, are most easily recalled and most likely to be over-reported.

The OFFHS study relied exclusively on maternal self-reports of adverse pregnancy outcomes with no medical or other validation. Generally, scientists place less confidence in reports of health outcomes that are not validated with medical records.

A confounding factor is a cause of a disease that is correlated with another exposure being studied. Failure to control confounding factors, especially those that are strong causes of a disease, can create spurious associations between benign exposures and diseases. In the Arbuckle study, there were at least three important potential confounding factors that were not controlled: history of previous spontaneous abortion, maternal age, and smoking. Even a weak correlation between these factors and use (or recall of use) of pesticides would produce spurious associations. In addition, in all three studies, the authors did not control the putative effect of one pesticide for the putative effects of other pesticides. So, for example, since farmers tend to use 4 or more pesticides each year, a disease that is associated with one pesticide will likely be associated with all, since their use patterns are correlated. In the

absence of an analysis that controls for multiple pesticides, the best that can be said is that the findings for any individual pesticide might be due to its correlation with another pesticide.

In summary, three publications based on data collected in the OFFHS found associations between several pesticides and various adverse reproductive outcomes. There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. Glyphosate was not significantly associated with adverse reproductive outcomes in two of these studies (Savitz *et al.* 1997, ASB2012-12022, Curtis *et al.* 1999, cited in ASB2012-11545). Glyphosate and other pesticides were weakly associated with spontaneous abortion in the study by Arbuckle (2001, ASB2012-11545). However, the author did not control for important personal confounding factors or for multiple exposures and no actual exposure data was used, casting doubt on the validity of the findings in this study.

Biomonitoring data for glyphosate, collected as part of the Farm Family Exposure Study (FFES), provide assurance that human health effects related to glyphosate exposure are very unlikely. In the FFES, researchers from the University of Minnesota collected 5 days of urine samples from 48 farm families before, during, and after a glyphosate application (Mandel *et al.*, 2005, ASB2012-11893, accepted for publication). Only 60% of farmers showed detectable exposure to glyphosate, with a 1 part per billion limit of detection, and the maximum estimated absorbed dose was 0.004 mg/kg (Acquavella *et al.*, 2004, ASB2012-11528). For farmers who apply glyphosate 10 times per year for 40 years, this maximum dose is more than 30,000-fold less than the EPA reference dose¹ of 2 mg/kg/day. For spouses, only 4% showed detectable exposures and the maximum systemic dose was 0.00004 mg/kg/day. Since glyphosate is not a reproductive toxic in high dose animal studies and since actual exposures on farms are so low, it is very unlikely that glyphosate would cause adverse reproductive outcomes for farmers or their spouses.

Author(s)	Year	Study title
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 Volume: 110 Pages: 441-449 ASB2012-11626

Abstract*

We previously demonstrated that the frequency of birth defects among children of residents of the Red River Valley (RRV), Minnesota, USA, was significantly higher than in other major agricultural regions of the state during the years 1989-1991, with children born to male pesticide applicators having the highest risk. The present, smaller cross-sectional study of 695 families and 1,532 children, conducted during 1997-1998, provides a more detailed examination of reproductive health outcomes in farm families ascertained from parent-reported birth defects. In the present study, in the first year of life, the birth defect rate was 31.3 births per 1,000, with 83% of the total reported birth defects confirmed by medical records. Inclusion of children identified with birth or developmental disorders within the first 3 years of life and later led to a rate of 47.0 per 1,000 (72 children from 1,532 live births). Conceptions in spring resulted in significantly more children with birth defects than found in

any other season (7.6 vs. 3.7%). Twelve families had more than one child with a birth defect (n = 28 children). Forty-two percent of the children from families with recurrent birth defects were conceived in spring, a significantly higher rate than that for any other season. Three families in the kinships defined contributed a first-degree relative other than a sibling with the same or similar birth defect, consistent with a Mendelian inheritance pattern. The remaining nine families did not follow a Mendelian inheritance pattern. The sex ratio of children with birth defects born to applicator families shows a male predominance (1.75 to 1) across specific pesticide class use and exposure categories exclusive of fungicides. In the fungicide exposure category, normal female births significantly exceed male births (1.25 to 1). Similarly, the proportion of male to female children with birth defects is significantly lower (0.57 to 1; p = 0.02). Adverse neurologic and neurobehavioral developmental effects clustered among the children born to applicators of the fumigant phosphine (odds ratio [OR] = 2.48; confidence interval [CI], 1.2-5.1). Use of the herbicide glyphosate yielded an OR of 3.6 (CI, 1.3-9.6) in the neurobehavioral category. Finally, these studies point out that a) herbicides applied in the spring may be a factor in the birth defects observed and b) fungicides can be a significant factor in the determination of sex of the children of the families of the RRV. Thus, two distinct classes of pesticides seem to have adverse effects on different reproductive outcomes. Biologically based confirmatory studies are needed.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Epidemiological study with some methodological / reporting deficiencies (selection of study subjects, no information about exposure duration, exposure concentration, pesticide use frequency).

Relevance of study:

Not relevant because of methodological deficiencies.

Klimisch code:

3

Additional comments:

Response 1 – summary from Mink et al. (2011) (ASB2012-11904)

Publication reports on different classes of pesticides and several birth defects and developmental outcomes.

Paternal use of glyphosate was associated with parent-reported ADD/ADHD in children (OR = 3.6). Six out of 14 children with parent reported ADD/ADHD also reported exposure to glyphosate.

Diagnoses of ADD/ADHD were not all confirmed. However, overall rate for the sample population (14/1532) was well below ADD/ADHD rates for the general population (7%).

Variables in statistical model analyses were not reported.

Response 2 – summary from Williams et al. (2012, ASB2012-12052)

Health data obtained via parent reporting for 695 families via written questionnaire and confirmed where possible.

Pesticide use information obtained initially via telephone then followed up by written questionnaire.

Reproductive health outcomes for births occurring between 1968 and 1998 were obtained for 1532 live births. Over half the births occurred prior to 1978, approximately 20 years after study initiation.

All pesticide use classes (herbicide only; herbicide and insecticide; herbicide, insecticide and fungicide; herbicide, insecticide and fumigant) were associated with birth defects.

Authors state neurobehavioral disorder would not be considered based lack consistent diagnoses. However, a detailed analysis was conducted for ADD/ADHD.

43% (6/14) parent reported children with ADD/ADHD were associated with glyphosate formulation use.

14 cases of ADD/ADHD reported out of 1532 live births, which is substantially lower than the diagnosed incidence of 7% for the general population.

No conclusions regarding glyphosate exposure and ADD/ADHD outcome can be drawn.

No other glyphosate specific data were reported.

Author(s)	Year	Study title
Garry, V.F., Holland, S.E., Erickson, L.L., Burroughs, B.L.	2003	Male Reproductive Hormones and Thyroid Function in Pesticide Applicators in the Red River Valley of Minnesota Journal of Toxicology and Environmental Health, Part A Volume: 66, Number: 11, Pages: 965-986 ASB2012-11627

Abstract*

In the present effort, 144 pesticide applicators and 49 urban control subjects who reported no chronic disease were studied. Applicators provided records of the season's pesticides used by product, volumes, dates, and methods of application. Blood specimens for examination of hormone levels were obtained in summer and fall. In the herbicide-only applicator group, significant increases in testosterone levels in fall compared to summer and also elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the fall were noted. With respect to fungicide use, in an earlier cross-sectional epidemiologic study, data demonstrated that historic fungicide use was associated with a significant alteration of the sex ratio of children borne to applicators. As before, among current study subjects it was noted that historic fungicide use was associated with increased numbers of girls being born. Lower mean total testosterone concentrations by quartile were also correlated with increased numbers of live-born female infants. A downward summer to fall seasonal shift in thyroid-stimulating hormone (TSH) concentrations occurred among applicators but not among controls. Farmers who had aerial application of fungicides to their land in the current season showed a significant shift in TSH values (from 1.75 to 1.11 mU/L). Subclinical hypothyroidism was noted in 5/144 applicators (TSH values >4.5 mU/L), but not in urban control subjects. Based on current and past studies, it was concluded that, in addition to pesticide exposure, individual susceptibility and perhaps economic factors may play a supporting role in the reported results.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Epidemiological study with some methodological / reporting deficiencies (e.g. selection of control subjects/samples, no details of exposure).

Documentation is insufficient for assessment.

Relevance of study:

Not relevant for glyphosate (due to reliability; in addition, no direct assessment of glyphosate exposure

was made).

Klimisch code:

3

Additional comments:

The publication brings little information on endpoints attributable to glyphosate.

Given the subjects were pesticide applicators, little can be drawn from the findings other than perhaps certain endpoints which may be associated with this specific occupation exposed to multiple chemical substances.

Of the 136 participants volunteering blood samples, only one individual (subject D) was noted with one abnormally high thyroid hormone levels associated with glyphosate use; thyroid stimulating hormone (FSH) was about double the normal range in the fall and thyroid stimulating hormone (TSH) higher than normal in the summer.

Another individual (subject E) had abnormally high TSH levels associated with multiple pesticide usage of 12 different active ingredients.

Author(s)	Year	Study title
Bell, E.M. Hertz-Picciotto, I. Beaumont, J.J.	2001	A Case-Control Study of Pesticides and Fetal Death Due to Congenital Anomalies Epidemiology Volume: 12, Number: 2, Pages: 148-156 ASB2012-11559

Abstract*

We examined the association between late fetal death due to congenital anomalies (73 cases, 611 controls) and maternal residential proximity to pesticide applications in ten California counties. A statewide database of all applications of restricted pesticides was linked to maternal address to determine daily exposure status. We examined five pesticide chemical classes. The odds ratios from logistic regression models, adjusted for maternal age and county, showed a consistent pattern with respect to timing of exposure; the largest risks for fetal death due to congenital anomalies were from pesticide exposure during the 3rd– 8th weeks of pregnancy. For exposure either in the square mile of the maternal residence or in one of the adjacent 8 square miles, odds ratios ranged from 1.4 (95 % confidence interval = 0.8 – 2.4) for phosphates, carbamates, and endocrine disruptors to 2.2 (95 % confidence interval = 1.3 – 3.9) for halogenated hydrocarbons. Similar odds ratios were observed when a more restrictive definition of nonexposure (not exposed to any of the five pesticide classes during the 3rd– 8th weeks of pregnancy) was used. The odds ratios for all pesticide classes increased when exposure occurred within the same square mile of maternal residence.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Epidemiological study with methodological deficiencies (e.g. glyphosate was included in the pesticide class of phosphates, thiophosphates, phosphonates; no differentiation between single and multiple exposures).

Relevance of study:

Not relevant (No glyphosate-specific results.)

Klimisch code:

3

Additional comments:**Response – summary from Williams et al. (2012, ASB2012-12052)**

Classes of pesticides were evaluated in this study, with glyphosate included as one of 47 active ingredients in the broad category of “phosphates/thiophosphates/phosphonates”.

Of the 47 active ingredients, many were organophosphate insecticide with known mammalian modes of action. The glyphosate mode of action is on the EPSPS enzyme in plants, which is not present in the animal kingdom.

Given the very low volatility of glyphosate and the low potential for inhalation exposures to aerosol sprays up to two miles away from the subjects, systemic doses to glyphosate would be considered negligible.

Mose et al., (2008, ASB2012-11914) demonstrated a low perfusion rate of glyphosate across the placenta. Coupled with the known low dermal and gastrointestinal absorption of glyphosate and the rapid elimination of systemic doses of glyphosate in the urine, human *in utero* exposures would be extremely limited.

The reported congenital anomalies associated with fetal death in Bell et al. (2001, ASB2012-11559) can in no way be linked to glyphosate exposure.

Author(s)	Year	Study title
Aris, A. Leblanc, S.	2011	Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reproductive toxicology Volume: 31, Pages: 528-533 ASB2012-11547

Abstract*

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and glufosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Exact levels of PAGMF, glyphosate or AMPA in the diets were not determined. It is not clear if the measured concentrations could have been resulted from other

exposure routes.

Relevance of study: Relevant with restrictions (Provides real life actual exposure concentrations in humans. Data are limited due to the absence of any information on applied pesticides, application rates, etc.).

Klimisch code: 3

Author(s)	Year	Study title
Benítez-Leite, S. Macchi, ML and Acosta, M.	2009	Malformaciones congénitas asociadas a agrotoxicos. Arch Pediatr Urug Volume: 80, Number: 3, Pages: 237-247 ASB2012-11563

Abstract*

Introduction: exposure to pesticides is a known risk for human health. This paper describes the relationship between parental exposure and congenital malformations in the newborn. Objective: to study the association between exposure to pesticides and congenital malformations in neonates born in the Regional Hospital of Encarnación, in the Department of Itapúa, Paraguay. Materials and methods: a prospective case-controlled study carried out from March 2006 to February 2007. Cases included all newborns with congenital malformations, and controls were all healthy children of the same sex born immediately thereafter. Births outside the hospital were not counted. Exposure was considered to be any contact with agricultural chemicals, in addition to other known risk factors for congenital defects. Results: a total of 52 cases and 87 controls were analyzed. The average number of births each month was 216. The significantly associated risk factors were: living near treated fields (OR 2,46, CI95% 1,09-5,57, $p<0,02$), dwelling located less than 1 km (OR 2,66, CI95% 1,19-5,97, $p<0,008$), storage of pesticides in the home (OR 15,35, CI95% 1,96-701,63, $p<0,003$), direct or accidental contact with pesticides (OR 3,19, CI95% 0,97-11,4, $p<0,04$), and family history of malformation (OR 6,81, CI95% 1,94-30,56, $p<0,001$). Other known risk factors for malformations did not show statistical significance. Conclusion: the results show an association between exposure to pesticides and congenital malformations. Further studies are required to confirm these findings.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable

Comment: Study design of epidemiological study for developmental toxicity insufficient for assessment, as well as methodological and reporting deficiencies (no assessment to which pesticides / active substances the mothers were exposed, use frequency not specified, selection of control group after study period is questionable, no information on exposure situation of mother for this control group assessed, etc.).

Relevance of study: Not relevant (The exposure to several pesticides was assessed in general, but no pesticide or active substance, including glyphosate, was specified or assessed).

Klimisch code:

3

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Description:	White solid
--------------	-------------

Lot/Batch #: Y04707/034
 Purity: 95.6% w/w
 Stability of test compound: The test substance was shown to be stable for the period of use.
 Vehicle: Deionised water
 Test animals:
 Species: Rats
 Strain: Alpk:APfSD (Wistar-derived)
 Source: [REDACTED]
 Age: At least 28 days
 Sex: Males and females
 Weight at dosing: ♂ 171.4 – 175.0 g; ♀ 144.6 – 148.7 g
 Acclimation period: Approx. 2 weeks
 Diet/Food: CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK), *ad libitum*, except 24 h prior dosing
 Water: Tap water, *ad libitum*
 Housing: In groups of five, separated by sex, in multiple rats racks.
 Environmental conditions: Temperature: 19 – 23 °C
 Humidity: 40 – 70 %
 Air changes: 25 – 30/hour
 12 hours light/dark cycle

In life dates: Not reported. The study was conducted during May and June 1995.

Animal assignment and treatment

In an acute neurotoxicity study groups of ten male and ten female Alpk:APfSD (Wistar derived) rats were administered with a single oral dose of 0, 500, 1000 and 2000 mg/kg bw glyphosate acid by gavage.

Dosing Formulation Analysis

Verification of the achieved concentrations was done with samples of each preparation. Homogeneity was determined with samples from the low to high dose levels. The chemical stability of glyphosate acid in water was also determined for all dose formulations over a period of 10 days.

Clinical observations

Clinical observations were made prior to administration and daily thereafter. Any abnormalities together with the observation of no abnormality detected were recorded.

Body weight

The body weight of each rat was recorded on Days -7 and -1, immediately before dosing (Day 1), approximately 6 hours after dosing (Day 1) and on Days 8 and 15.

Food consumption

Food consumption for each cage of rats was recorded throughout the study and calculated on a weekly basis.

Functional Observational Battery

Prior to the start of treatment (Week -1) and on Day 1, 8 and 15, all animals were observed for signs of functional/behavioural toxicity. Detailed clinical assessments and functional performance tests were performed together with an assessment of sensory reactivity to different stimuli. Locomotor activity was also assessed at these time points.

Sacrifice and pathology

At scheduled termination, 5 rats/sex/group designated for neuropathology were sacrificed. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve. Neuropathological examination was performed on control and highest dose group animals only.

Statistics

Analyses of variance and covariance were carried out using the GLM procedure in SAS (1989). Least-squares means for each group were calculated using LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squared mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

Results and discussion

Dosing formulation analysis: The achieved concentrations of glyphosate acid in water were within 3 % of the nominal levels. The homogeneity was considered acceptable, with a deviation from the overall mean values of approximately ± 8 %. The chemical stability was considered satisfactory.

Mortality and clinical observations:

Two females receiving 2000 mg/kg bw glyphosate acid showed subdued behaviour, decreased activity, hunched posture, sides pinched in, tip-toe gait and hypothermia on the day of administration. One of these animals died on the subsequent day. The other one together with an additional female which showed diarrhoea on the day of administration regained full recovery the subsequent day.

One female receiving 500 mg/kg bw, was found dead approximately 6 h after administration. In the absence of any treatment-related clinical signs prior to death, and because no deaths were observed at the intermediate dose level of 1000 mg/kg bw, the death of this animal was considered not to be treatment related.

Distension of the abdomen was recorded for several males from all treated groups on the day of administration. However, in the absence of any dose relationship, this was not considered to be treatment-related.

Body weight:

No treatment-related effects were observed.

Food consumption

During Week 1, mean food consumption was lower in animals receiving 2000 mg/kg bw glyphosate acid compared to controls, although the difference did attain statistical significance only in females (see Table B.6.7-1). There was no evidence of treatment-related effects in animals receiving 500 or 1000 mg/kg bw.

Table B.6.7-1: Intergroup comparison of food consumption (g/rat/day) during Week 1

Dose level of glyphosate (mg/kg bw)			
0 (control)	500	1000	2000
Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Males			
29.9 ± 0.7	29.0 ± 0.1	30.1 ± 0.4	28.4 ± 0.2
Females			
22.4 ± 1.0	22.2 ± 0.2	22.8 ± 0.3	20.6* ± 0.3

* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)

Functional observation battery: Examinations of the functional observational battery did not identify any conclusive treatment- and dose-related effects.

Necropsy: No macroscopic findings were detected.

Histopathology: No microscopic findings were considered to be treatment-related.

Conclusion by the Notifiers

Based on the study results the NOAEL for acute neurotoxicity, following single oral administration of glyphosate acid is 2000 mg/kg bw.

RMS comments:

The study is considered acceptable. Its evaluation as provided in the dossier is agreed with. There was no evidence of specific neurotoxicity up to the highest single dose of 2000 mg/kg bw. Clinical signs indicative of acute toxicity occurred in few animals at this dose and included one death. Thus, the NOAEL for systemic toxicity was 1000 mg/kg.

Subchronic neurotoxicity study in rats [REDACTED], 1996)

Reference: IIA, 5.7.4/01

Report: [REDACTED] 1996, Glyphosate acid: Subchronic neurotoxicity study in rats

Data owner: Syngenta

Report No.: CTL/P/4867

Date: 1996-03-11

unpublished,

ASB2012-11501

Guidelines: Study was pre-guideline, but satisfies in general the requirements of OECD 424 (1997)

Deviations: None

GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 1995-04-25 to August 1995

Materials and methods

Test material:

Identification: Glyphosate acid (technical)

Description: White solid

Lot/Batch #: P24

Purity: 95.6 %

Stability of test compound: Confirmed for the study period

Vehicle: Plain diet

Test animals:

Species: Rats

Strain: Alpk:APfSD

Source:

Age: At least 6 weeks

Sex: male and female

Weight at dosing: ♂ 215.0 – 218.6 g (mean); ♀ 173.5 – 178.8 g (mean)

Acclimation period: Approximately 2 weeks

Diet/Food: CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum* (except up to 24 hours prior to dosing)

Water: Tap water, *ad libitum*

Housing: Four per cage per sex in stainless steel cages (26.5 x 50.0 x 20.7cm)

Environmental conditions: Temperature: 19-23 °C
 Humidity: 40-70 %
 Air changes: 25-30/hour
 Photoperiod: 12 hours light/dark cycle

In life dates: 1995-05-09 to August 1995

Animal assignment and treatment:

In a subchronic neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks. (equivalent to mean achieved dose levels of 0, 155.5, 617.1 and 1546.5 mg/kg bw/day for males, and 0, 166.3, 672.1 and 1630.6 mg/kg bw/day for females) glyphosate technical.

All diets were based on CT1 diet supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK. The experimental diets were prepared in 30 kg batches by direct addition of the test substance to 30 kg of CT1 diet and mixing thoroughly. The diets were stored at room temperature until required for use.

Samples from all dietary levels (including controls) were taken at intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in

CT1 diet was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet, under the conditions of storage used on this study, was determined for 2000 ppm and 20000 ppm diets prepared for use on a concurrent 1 year feeding study in the rat in the same laboratory.

Clinical observations

A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded. A detailed physical examination was performed on each rat prior to start of treatment, and at weekly intervals thereafter.

Functional observational battery (FOB)

Prior to the start of treatment and during Weeks -1, 5, 9 and 14, all animals were observed for signs of functional/behavioural toxicity. The assessment involved observations in the home cage and/or while the rat was moving freely in a standard arena followed by manipulative/in hand tests. Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. The examinations included quantitative assessments of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hind limb grip strength). The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour; reactivity to stimuli; changes in level of arousal; sensorimotor responses; alterations in respiration.

Locomotor activity

Locomotor activity was monitored by an automated activity recording apparatus. All animals were tested at weeks -1, 5, 9 and 14. Each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimise disturbances.

Body weight

Individual body weights were recorded in week -1, immediately prior to treatment), at weekly intervals thereafter, and at necropsy.

Food consumption and compound intake

Food consumption was recorded as required for each cage group throughout the study and calculated on a weekly basis. Food utilisation and compound intake were calculated.

Water consumption

Not reported.

Ophthalmoscopic examination

Not performed. However, ophthalmological data are available from other repeated dose studies.

Sacrifice and pathology

At the scheduled termination, all main study animals not required for neuropathology, were killed by overexposure to rising concentrations of carbon dioxide gas and were discarded without examination.

At termination, the six rats/sex/group designated for neuropathology were deeply anaesthetised with intraperitoneal sodium pentobarbitone and killed by whole body perfusion fixation with modified Karnovsky's solution. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve.

Brain weight, brain length and brain width were determined.

Submitted tissues were processed as follows: brain (seven levels including the cerebral cortex, the hippocampus, the cerebellum, the pons and medulla), dorsal root ganglia and spinal roots from cervical and lumbar regions of the cord after decalcification, and gastrocnemius muscle from rats receiving either control diet or diet containing 20000 ppm glyphosate acid were routinely processed, paraffin wax embedded and 5µm thick sections were cut and then stained with haematoxylin and eosin. Sections of brain and cord were in the transverse plane.

The Gasserian ganglion, sciatic nerve, spinal cord (cervical and lumbar portions), sural and tibial nerve from control and high dose group rats were processed and then embedded in Araldite. Semi-thin sections were cut and then stained with toluidine blue. For bilateral tissues only the left was processed. All tissues were sectioned in the transverse plane except the sciatic nerve which was sectioned in both the transverse and the longitudinal plane.

Neuropathological examination was performed on control and highest dose group animals only. All sections were examined by light microscopy.

Statistics

All data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter using the GLM procedure in SAS (1989).

The levels of probability chosen as significant different from control were $p < 0.01^{**}$ and $p < 0.05^{*}$ (Student's t-test, two-sided).

Results and discussion

Dosing formulation analysis: The achieved mean concentrations of Glyphosate acid in diet were within 4 % of the nominal levels, with individual values being within 15 % of nominal. There were considered acceptable. The homogeneity of the low- and high-dose diets was considered acceptable, with a deviation from the overall mean values of ± 4 %. The chemical stability was considered satisfactory.

Mortality: No deaths occurred during the study.

Clinical observations: There were no treatment-related clinical signs of toxicity.

Functional observational battery (FOB)

There were no clinical signs that could be attributed to administration of glyphosate acid.

There was an apparent increase in the incidence of miosis and decreased pupil response to light in males receiving 20000 ppm. However, as these signs were seen for several of these males pre-experimentally and were also present at a similar incidence in females with no obvious relationship to treatment, this was considered to be incidental and unrelated to administration of glyphosate acid.

Landing Foot Splay Measurements

There was no evidence of any treatment-related effect on landing foot splay.

Time to Tail-Flick

There was no evidence of any treatment-related effect on time to tail-flick.

Grip Strength Measurements

There was no evidence of any treatment-related effect on forelimb or hind limb grip strength.

Motor activity

There was no evidence of any treatment-related effect on locomotor activity.

During week 5, slightly reduced locomotor activity was recorded on occasions for females receiving 20000 ppm. However, in the absence of any treatment-related effects on motor activity for these animals at other time points during the study, this is considered to be incidental and unrelated to administration of glyphosate acid.

Table B.6.7-2: Selected motor activity findings

		Dietary concentration (ppm)							
		Males				Females			
Week	Assessment period (min)	0	2000	8000	20000	0	2000	8000	20000
5	1-50	388.7	472.1	335.6	384.4	441.2	379.3	457.8	359.3
9	1-50	304.7	413.4*	298.4	327.3	512.3	488.9	555.1	557.0
14	1-50	299.4	395.1	292.2	372.8	553.0	512.7	569.3	514.7

* Statistically significant difference from control group mean at the 5% level (Student's t-test, 2-sided)

** Statistically significant difference from control group mean at the 1% level (Student's t-test, 2-sided)

Body weight: Group mean bodyweight for males receiving 20000 ppm was statistically significantly lower than that of controls throughout the study. At week 14, group mean bodyweight for these animals was 92.8 % that of controls, equating to a reduction in bodyweight gain of approximately 12 %.

Group mean body weight for males receiving 8000 ppm was also marginally lower than that of controls from weeks 6 to 14. However, these differences did not attain statistical significance and were considered too small to be of biological importance.

For males receiving 2000 ppm, and for females at all dose levels, mean body weight was essentially similar to that of concurrent controls throughout the study.

Table B.6.7-3: Intergroup comparison of body weights (g)

Week	Dietary concentration (ppm)							
	Males				Females			
	0	2000	8000	20000	0	2000	8000	20000
1	216.0	217.0	218.6	215.0	173.5	178.8	175.6	175.3
2	263.5	264.7	264.9	254.6**	192.7	200.6	196.1	194.3
4	338.2	340.7	339.6	323.7*	214.3	228.3**	224.9**	219.2
8	440.7	440.1	429.1	405.8**	253.6	262.1	260.4	255.4
14	534.7	532.8	526.5	496.1**	285.1	291.5	287.9	281.0

* Statistically significant difference from control group mean at the 5 % level (Student's t-test, 2-sided)

** Statistically significant difference from control group mean at the 1 % level (Student's t-test, 2-sided)

Food consumption and compound intake: There were no effects on food consumption. The efficiency of food utilisation for males receiving 20000 ppm was statistically significantly lower than that of concurrent controls during weeks 1 to 8. There were no changes in the efficiency of food utilisation for males receiving 2000 or 8000 ppm or for females from all treated groups.

Table B.6.7-4: Intergroup comparison of food utilisation (g growth/100 g food)

Week	Dietary concentration (ppm)							
	Males				Females			
	0	2000	8000	20000	0	2000	8000	20000
1-4	18.13	17.16	16.94	16.28*	9.42	9.73	9.36	9.61
5-8	11.52	10.69	10.35	9.93*	5.99	5.55	5.39	5.70
1-13	12.00	11.45	11.38	10.87**	6.08	6.03	6.06	5.96

* Statistically significant difference from control group mean at the 5 % level (Student's t-test, 2-sided)

** Statistically significant difference from control group mean at the 1 % level (Student's t-test, 2-sided)

The mean doses received for males and females respectively were 155.5, 617.1, 1546.5 and 166.3, 672.1, 1630.6 mg glyphosate acid/kg/day at dose levels of 2000, 8000 and 20000 ppm, respectively

Brain measurements: There was no evidence of any effects on brain weight, length or width.

Necropsy: There were no macroscopic findings that were considered to be attributable to treatment.

Histopathology: There were no microscopic findings in the peripheral or central nervous system that were considered to be attributable to treatment.

Conclusion by the Notifiers

Dietary administration of glyphosate acid to rats for a period of ninety consecutive days at dietary concentrations of up to 20000 ppm produced evidence of toxicity in the form of reduced growth and reductions in food utilisation for males. Comprehensive histopathological evaluation of the nervous system showed no evidence of any changes in the peripheral or central nervous system which could be attributed to administration of glyphosate acid.

The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm, corresponding to 1546.5 / 1630.6 mg/kg bw/day for males and females, respectively.

RMS comments:

The study is considered acceptable. The conclusion is agreed with. No evidence of a neurotoxic potential was obtained up to the the highest dose of 20000 ppm. The NOAEL for systemic toxicity was 8000 ppm (617 mg/kg bw/day) in males, based on lower body weight (gain) and impaired food utilisation, and 20000 ppm (1631 mg/kg bw/day) in females, i.e. no effects were noted at the top dose level.

Delayed neurotoxicity study in chicken (Johnson, 1996)

Reference: Not applicable since study was not part of the dossier

Report: [REDACTED] 1996, Glyphosate Acid: Acute delayed neurotoxicity study in the domestic hen.
[REDACTED]
Data owner: Syngenta (study was not part of the dossier but submitted to RMS for other purposes before)
Report No.: CTL/C/3122
Date: 1996-08-23
unpublished,
ASB2013-9828

Guidelines: U.S. EPA Subdivision F 81-7

Deviations: None

GLP: Yes

Acceptability: The study is considered acceptable by RMS.

Materials and methods

Test material:

Identification: Glyphosate acid (technical)

Description: White solid

Lot/Batch #: P24

Purity: 95.6 %

Stability of test compound: Confirmed for the study period

Vehicle: Distilled water

Positive control substance:

Identification: Tri-ortho-cresylphosphate (TOCP)

Lot/Batch: 143-41C

Purity: 99.0 %

Expiry date: November 1997

Supplier: Chem Services Inc.

Vehicle: Corn oil

Test animals:

Species: Chicken

Strain: Lohmann Brown (a hybrid brown laying strain)

Source: [REDACTED]

Age: Approx. 12 months

Sex: Female

Weight at dosing: 1927 – 2215 g (range)

Acclimation period: Approximately 2 weeks

Diet/Food: HRC layer ration in pellet form (Parker Bros. Ltd., Lark Mills, Suffolk, UK), *ad libitum* (except overnight starvation prior to dosing)

Water: Tap water, *ad libitum*

Housing: Floor pens (galvanised steel, concrete floor) measuring 1.8 x 1.4 m with up to (not further specified) hen from the same group

Environmental conditions: Temperature: 15-17 °C
Humidity: 79 % (mean)
Air changes: not given in the report but ventilation considered “adequate” by study author
Photoperiod: 12 hours light/dark cycle

In life dates: 1996-01-09 to 1996-02-14

Animal assignment and treatment:

20 hen were administered glyphosate acid as a single dose of 2000 mg/kg bw by oral gavage. 12 birds were employed as positive controls and received a single dose of 1000 mg TOCP/kg bw. The negative control group consisted also of 12 hen and received once distilled water also by gavage. The same volume of 10 mL/kg bw was applied to all chicken. Treatment was followed by an observation period of 21 or 22 days.

Clinical observations

A check for mortality, clinical signs of toxicity, ill health and behavioural changes was made twice daily on all animals.

Ataxia assessment

Following treatment, hen were examined daily for signs of (delayed) ataxia.

Body weight

Individual body weights were recorded weekly.

Food consumption and compound intake

No information given.

Water consumption

Not reported.

Clinical pathology

3 pre-determined chicken from each group were sacrificed 48 hours after dosing to determine brain cholinesterase, brain neuropathy target esterase and lumbar spinal cord neuropathy target esterase (NTE) activities

Sacrifice and pathology

At the scheduled termination, 6 hen from each group were selected for necropsy and histopathological examinations. Whereas in the negative control and glyphosate-treated

groups the first six birds in numerical order (because of the absence of clinical signs) were employed, care was taken in the TOCP-treated group to include all animals that had shown clinical ataxia. The remaining hen from all three groups were killed and discarded.

At termination, after perfusion through the heart with fixative, head and spinal column (with brain and spinal cord exposed but left in place) and dissected sciatic nerves (including tibial branches) from the six hen/group designated for neuropathology were taken and stored. The following tissues were used to take samples for histological examination: brain (forebrain, mid and hindbrain), spinal cord (upper and lower cervical, mid-thoracic and lumbo-sacral parts), sciatic nerve (proximal and distal, above knee), tibial nerve. One transverse and two longitudinal sections were performed at each level.

Statistics

Apparently, no statistical analysis was necessary since the results were quite clear and number of animals limited.

Results and discussion

Mortality: There were two unscheduled deaths during the study.

- In the test group receiving glyphosate acid, one bird was found dead on day 10 after dosing. This hen had not exhibited any signs of toxicity prior to death. The cause of death was apparently not elucidated but if the singularity of this case (1/20) and the absence of clinical signs is taken into consideration, the opinion of the study author can be agreed with that mortality was unrelated to treatment.
- In the positive (TOCP) control group, one bird had been severely pecked by other hen and was sacrificed on humane grounds, apparently during the acclimation period yet.

Clinical observations: In the glyphosate-treated and negative control groups, there were no was a common finding (see below). In addition, one more bird in this group was hurt by other hen by pecking and had to be treated by applying Stockholm tar to the wounds.

Ataxia assessment

Ataxia was confined to the positive control group receiving 1000 mg TOCP/kg bw. 5 of 11 hen were affected. Signs occurred for the first time between post-observation days 11 and 21 and the severity of ataxia was variable.

Body weight

Group mean body weight increased in the glyphosate-treated and negative control groups but weight loss was observed in the positive controls receiving TOCP.

Clinical chemistry

In line with ataxia observations, NTE levels in brain and spinal cord were clearly reduced in the positive control group (by 84% for brain and by 78% for spinal cord as compared to negative control group) but no effect was seen in the group receiving glyphosate. A very low reduction of brain cholinesterase (6% less than in negative control) was seen in the hen that had received glyphosate. In the positive control group, the decrease in brain cholinesterase activity accounted for 19%. In the study report, the lack of statistical significance for the latter findings is emphasised but it is doubted if meaningful statistical analysis was possible with only three birds per group under investigation. Taking into account the very low difference to

negative control birds and the fact that glyphosate is not known to inhibit cholinesterases, a treatment-related effects is not likely.

Necropsy: There were no macroscopic findings that were attributable to treatment.

Histopathology: The evaluation of histological findings is complicated by the fact that axonal degeneration in the spinal cord and peripheral nerves were observed in all three groups in nearly all birds suggesting high background incidence. In the TOCP-treated group, the cerebellum was also affected in five out of 6 animals (as compared to only one bird in the glyphosate group). Furthermore, axonal degeneration in general was more severe in the positive control group.

Conclusion (RMS)

Based mainly on the occurrence of ataxia in the positive control group but not in chicken receiving glyphosate and the related reduction of NTE activity after dosing with TOCP but not with glyphosate, there is no potential of glyphosate to cause delayed neuropathy.

B.6.7.1 Published data on neurotoxicity

Several publications over the last decade have evaluated glyphosate with respect to neurotoxicity endpoints. Three papers report a total of two human cases of Parkinson's disease. The first case followed acute exposure to a glyphosate formulation while spraying a garden (Barbosa et al., 2001, ASB2012-11557; da Costa et al, 2003, ASB2012-11598). The second case reported chronic exposures to a factory worker in China, where a variety of pesticides including glyphosate were produced (Wang et al, 2011, ASB2012-12047). Several questions arise in attempting to link glyphosate exposures with each case of Parkinson's disease. Firstly, significant systemic exposures to glyphosate in each instance are questionable, given the poor dermal absorption and low volatility of the compound. Secondly, if glyphosate was a causative agent of this fairly common disease, a significant number of cases associated with either acute and/or chronic exposures would be evident. Glyphosate formulations are sometimes readily accessible for suicide attempts, which are usually unsuccessful, as less than 10% of glyphosate self administered ingestions result in death. No reports of Parkinson's disease in survivors following very acute ingestions of glyphosate products have been documented. Glyphosate has been manufactured and widely used in agriculture and consumer markets for approximately 40 years, so a single case of a pesticide factory worker developing Parkinson's disease, while unfortunate, does not constitute cause and effect; there is no evidence of a higher frequency of Parkinson's disease in glyphosate production workers.

Multiple long term animal studies with glyphosate have failed to demonstrate any evidence of neurotoxicity and certainly have not shown evidence of Parkinson's-like abnormalities. While some studies have suggested statistical associations with general pesticide exposure or general insecticide or herbicide exposure (Engel et al., 2001, ASB2012-11612), there is no evidence suggesting a specific association between glyphosate and Parkinson's disease. In the largest study to date of US Farmers (Agricultural Health Study), no increased risk of Parkinson's disease was found in association with reported glyphosate use (Kamel et al., 2007, ASB2012-11862). Human non-cancer epidemiologic outcomes related to glyphosate have recently been reviewed (Mink et al. 2011, ASB2012-11904), and there is no convincing

evidence for an increased incidence of Parkinson's disease or other neurological disorders in individuals reporting glyphosate exposure.

Several publications open with the premise that pesticide exposures are linked with Parkinson's disease, and then proceed to report *a priori* research linking glyphosate with a measurable endpoint. This endpoint is then extrapolated to link with Parkinson's disease in humans. Despite the lack of compelling human associations between glyphosate exposure and Parkinson's disease, such research continues to be published. Astiz et al., (2009, ASB2012-11549), Negga et al. (2011, ASB2012-11923) and Gui et al. (2012, ASB2012-11835) all conducted glyphosate research in the above mentioned manner, all in very different test systems. Negga et al. (2011, ASB2012-11923) notes neurodegeneration in *Caenorhabditis elegans* worms following exposure to glyphosate (trimesium form, which has a different toxicology profile than glyphosate) uses concentrations equal to the LD25, LD50 and LD75, or actual concentrations of glyphosate of 3 to 10 percent, i.e.- the high concentration is approximately 10-fold higher than concentrations applied directly in the field. The relevance of such high-dose exposures to the trimesium salt in this experimental model to human Parkinson's disease is highly questionable and irrelevant to the Annex 1 renewal of glyphosate technical acid. Astiz et al. (2009, ASB2012-11549) and Gui et al. (2012, ASB2012-11835) both affirm their test models (in rats and in PC-12 cells respectively) for evaluating neurodegenerative disorders, then directly link their research results to Parkinson's disease in humans; these two studies are addressed below.

Cole et al. (2004, ASB2012-11594) evaluated 15 different pesticides for neurotoxic end points in *C. elegans* with analytical grade active ingredients, noting reduced cholinesterase for pesticides with this mode of action, but not glyphosate. Interestingly, the authors report a low pH effect resulting in reduced cholinesterase activity in the high dose of glyphosate and a plant growth promoter. Glyphosate formulations contain salt forms of glyphosate, not the technical acid and thus do not have a low pH. Additionally, human incidents of self induced glyphosate poisonings do not report the common symptoms of acute acetylcholinesterase inhibition; salivation, lacrimation, urination and defecation (SLUD).

Author(s)	Year	Study title
Barbosa, E.R. Leiros da Costa M.D. Bacheschi, L.A. Scaff M.	2001	Parkinsonism After Glycine-Derivate Exposure Movement Disorders Volume: 16, Number: 3, Pages: 565-568 ASB2012-11557

Abstract*

This 54-year-old man accidentally sprayed himself with the chemical agent glyphosate, a herbicide derived from the amino acid glycine. He developed disseminated skin lesions 6 hours after the accident. One month later, he developed a symmetrical parkinsonian syndrome. Two years after the initial exposure to glyphosate, magnetic resonance imaging revealed hyperintense signal in the globus pallidus and substantia nigra, bilaterally, on T2-weighted images. Levodopa/benserazide 500/125 mg daily provided satisfactory clinical outcome.

* Quoted from article

Klimisch evaluation

Reliability of study: Not assignable
 Comment: Medical case report, single incident
 Relevance of study: Relevant with restrictions (Data are limited due to the absence of any information on purity and application concentrations of glyphosate formulation, as well as co-formulations.)
 Klimisch code: 4

Author(s)	Year	Study title
Wang, G., Xiao-Ning, F., Yu-Yan, T., Qi, Ch., Shen-Di, CH.	2011	Parkinsonism after chronic occupational exposure to glyphosate. Parkinsonism and related disorders ASB2012-12047

Abstract*

Here we report a patient with parkinsonism following chronic occupational exposure to glyphosate. A previously healthy 44-year-old woman presented with rigidity, slowness and resting tremor in all four limbs with no impairment of short-term memory, after sustaining long term chemical exposure to glyphosate for 3 years as a worker in a chemical factory. The chemical plant produced a range of herbicides including: glyphosate, gibberilins, and dimethyl hydrogen phosphite; however, the patient worked exclusively in the glyphosate production division. She only wore basic protection such as gloves or a face mask for 50 h each week in the plant where glyphosate vapor was generated. She frequently felt weak. ... Physical examination revealed a parkinsonian syndrome. ...

We cannot exclude the coincidence of idiopathic PD with exposure to glyphosate on our patient. ...

* Quoted from article

Klimisch evaluation

Reliability of study: Not assignable
 Comment: Medical case report, single incident
 Relevance of study: Relevant with restrictions
 Klimisch code: 4

Author(s)	Year	Study title
Astiz, M. de Alaniz, M.J. Marra, C.A.	2009	Effect of pesticides on cell survival in liver and brain rat tissues Ecotoxicology and Environmental Safety Volume: 72, Pages: 2025-2032, ASB2012-11549

Abstract*

Pesticides are the main environmental factor associated with the etiology of human neurodegenerative disorders such as Parkinson's disease. Our laboratory has previously demonstrated that the treatment of rats with low doses of dimethoate, zineb or glyphosate alone or in combination induces oxidative stress (OS) in liver and brain. The aim of the present work was to investigate if the pesticide-induced OS was able to affect brain and liver cell survival. The treatment of Wistar rats with the pesticides (i.p. 1/250 LD50, three times a week for 5 weeks) caused loss of mitochondrial transmembrane potential and cardiolipin content, especially in substantia nigra (SN), with a concomitant increase of fatty acid peroxidation. The activation of calpain apoptotic cascade (instead of the caspase-dependent pathway) would be responsible for the DNA fragmentation pattern observed. Thus, these results may contribute to understand the effect(s) of chronic and simultaneous exposure to pesticides on cell survival.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Unsuitable test system (i.p exposure route is not relevant for human exposure). No information on purities of test substances used. Small group size (4 males/dose group), reporting deficiencies
Relevance of study:	Not relevant (intraperitoneal injection is a non-relevant route of exposure for humans)
Klimisch code:	3

Additional comments

This non-guideline study utilized very small group numbers (4 rats/group) and therefore is not sufficiently robust to appropriately identify changes attributable to the test material administration.

The test materials are not well described, without indication of whether a glyphosate salt form or acid was used and purity was not reported.

The publication focuses on the post necropsy data analysis and reporting. Data on animal husbandry, clinical observations, feed and water intake, weekly body weight were not reported, but the authors note there were no adverse observations.

No statistically significant effects were noted for liver endpoints, yet the liver is in close proximity to test material administration via intraperitoneal injection.

Statistically significant effects were noted for brain tissue endpoints in the substantia nigra and cerebral cortex. However, there is a lack of biological plausibility for brain exposures to glyphosate, given the necessity to pass the blood-brain barrier and the known rapid elimination kinetics of this polar molecule via urine.

Author(s)	Year	Study title
Gui, Y.X., Fan, X.N., Wang, H.M., Wang, G., Chen, S.D.	2012	Glyphosate induced cell death through apoptotic and autophagic mechanisms. Neurotoxicology and teratology Volume: not specified (<i>accepted manuscript</i>) Pages: not specified ASB2012-11835

Abstract*

Herbicides have been recognized as the main environmental factor associated with human neurodegenerative disorders such as Parkinson's disease (PD). Previous studies indicated that the exposure to glyphosate, a widely used herbicide, is possibly linked to Parkinsonism, however the underlying mechanism remains unclear. We investigated the neurotoxic effects of glyphosate in differentiated PC12 cells and discovered that it inhibited viability of differentiated PC12 cells in dose- and time-dependent manners. Furthermore, the results showed that glyphosate induced cell death via autophagy pathways in addition to activating apoptotic pathways. Interestingly, deactivation of Beclin-1 gene attenuated both apoptosis and autophagy in glyphosate treated differentiated PC12 cells, suggesting that Beclin-1 gene is involved in the crosstalk between the two mechanisms.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Documentation insufficient for assessment (not clearly stated dose levels and duration of exposure, as well as treatment conditions for all tests. In addition, tested doses were much higher than real in vivo concentrations).

Relevance of study:

Not relevant (Due to reliability)

Klimisch code:

3

Additional comments:

In this paper, the authors apply glyphosate to adrenal cancer cells in culture at concentrations sufficient to cause cell death. Two major interacting pathways leading to cell death (autolysis and apoptosis) are evaluated, and the results are hardly surprising - the cells do indeed die via known mechanisms leading to cell death. The authors use these observations, and the fact that Parkinson's disease involves the death of certain nerve cells in the brain, to try and create a link between glyphosate and Parkinson's disease. There are, however, many problems with this extrapolation.

The cells used are not the neurons involved in Parkinson's, but rather a cell line derived from an adrenal gland cancer (pheochromocytoma), and the doses used are very high- the high dose killed nearly 50 % of cells in 72 hours, and the low dose was 1/4 this level. The high dose equates to approximately 1/10 the concentration applied directly in the field, and is far higher than any internal glyphosate concentration that could ever occur following glyphosate use. A sufficiently high dose of every substance will kill cells - but this does not mean that every substance causes Parkinson's disease.

Unprotected cells in culture are highly susceptible to changes in pH and other non-specific effects, and it is not clear that the researchers assessed or accounted for these possible effects.

This being said, the concentrations of glyphosate used (40 mM) are known to kill other cell

types in culture (Heu et al., 2012, ASB2012-11844) via induction of apoptosis. Thus, no particular specificity or neuronally-specific susceptibility exists for the cell line tested. While 40 mM glyphosate is toxic to cells in culture, the LD₅₀ in rodents is over 5000 mg/kg and *C. elegans* will have a 25 % survival following exposure to a 10 % solution of glyphosate. *In-vitro* results do not appear to reflect *in vivo* events.

Anadon et al. (2009, ASB2012-11542) dosed rats with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved glyphosate peak modeled plasma concentrations of approximately 5 ug/mL (5 ppm). Assuming linear kinetics, the current maximum allowable EU daily intake (0.3 mg/kg/day) would give an approximated blood concentration of 0.17 ppm (170 ppb). This is conservative, as McQueen et al (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4 % of the current European acceptable daily intake.

The lowest glyphosate concentration used in this experiment is 5mM (830 ppm), or 5000 times higher than the estimated blood concentration following the current EU maximum allowable daily exposure. It is also 166 times higher than the concentrations Anadon et al. (2009, ASB2012-11542) achieved using doses of 400 mg/kg glyphosate. In short, the concentrations used in this work are massively higher than any concentration in blood (let alone brain tissue) that can be achieved following normal human exposures.

B.6.8 Further toxicological studies

Introduction into this chapter by the RMS

The metabolite aminomethyl phosphonic acid (AMPA) was extensively investigated for acute and subchronic effects, mutagenicity and developmental toxicity. Most of these studies had been submitted for the previous EU evaluations of either glyphosate or glyphosate-trimesium yet and were re-evaluated now by the RMS for reliability and acceptability. Previously known toxicity and genotoxicity studies of insufficient quality (from a today's perspective) have been deleted (██████████ 1973, TOX9552394; ██████████ 1978, TOX9552399; ██████████ 1991, TOX9552415; ██████████ 1980, TOX9552408; ██████████ 1991, TOX9552404). The same holds true for a brief information concerning kinetics and (absent) metabolism of AMPA (Colvin, ██████████, 1973, TOX9552354). Main exclusion criteria were lacking information on purity of AMPA, severe reporting deficiencies, a too low number of animals employed or the absence of relevant examinations such as histopathology that are usually required. Range-finding studies have not been considered so far a subsequent definitive main study is available.

Meanwhile, a few more studies on acute toxicity in rats and mice, on skin sensitisation and genotoxicity have been submitted that were not subject to former EU evaluation and, therefore, are reported in detail below.

All valid studies with AMPA are compiled and summarised in Table B.6.8-1.

Other metabolites of glyphosate have not been addressed in the GTF dossier from a toxicological point of view and, indeed, may be not relevant when only the intended applications and the representative formulation are taken into consideration.

However, the metabolite N-acetyl glyphosate is newly proposed to be part of the residue definition that will occur in some genetically modified plants after application of glyphosate (see also EFSA, 2009; ASB2012-3480). Toxicological studies with this metabolite have not been submitted as part of the GTF dossier to support new approval of glyphosate in the EU but were subject to a previous (2008) evaluation by the RMS that was performed in order to set import tolerances for glyphosate in soy beans and maize from genetically modified plants. This evaluation may be included in this re-evaluation of glyphosate on request by EFSA or other MS.

Sometimes, the minor metabolite N-methyl-N-(phosphonomethyl)glycine was detected. An acute oral toxicity in rats with that metabolite was described in the 1998 DAR (██████████ 1991, TOX9552398). In this (acceptable upon re-evaluation) study, no deaths occurred at the limit dose of 5000 mg/kg bw but some clinical signs were observed.

The second sub-section deals with possible effects on farm animals. In contrast to most other active substances, experimental toxicological studies in goats and cows are available with glyphosate and were described in detail by GTF on request of the RMS. Below each study description, the RMS conclusions and an assessment of validity/acceptability of this study may be found.

Recent findings of glyphosate in the urine of cows are reported and have been put into perspective by comparing the estimated systemic dose with proposed ADI and with the NOAELs/LOAELs in the abovementioned studies in cattle. Furthermore, a number of recent publications is discussed in which a possible impact of glyphosate on gut microflora of farm animals has been investigated.

A large number of studies on toxicity of glyphosate was published since 2000. Most of these studies are presented in the chapters on genotoxicity, carcinogenicity, reproductive toxicity and neurotoxicity of this report because they are discussed there in context with these endpoints. However, some additional studies that could not be allocated to a certain endpoint are presented at the end of this chapter.

B.6.8.1 Toxicity of the metabolite AMPA

Table B.6.8-1: Summary of toxicological studies with AMPA

Reference; study identification, owner	Type of study; Species, strain	AMPA purity [%]	Exposure conditions / test method; dose levels	Results
██████████ 1993; TOX9552395; Cheminova	Acute oral toxicity Rat, SD	99.2	Limit test (m/f)	LD ₅₀ > 5000 mg/kg bw; some general and gastrointestinal clinical signs observed
██████████ 1988; TOX9500044; Syngenta	Acute oral toxicity Mouse, ICR	99.33	Limit test (m/f)	LD ₅₀ > 5000 mg/kg bw, no evidence of toxicity
██████████ 1988; TOX9500044; Syngenta	Acute oral toxicity Rat, Wistar	> 99	Limit test (m/f)	LD ₅₀ > 5000 mg/kg bw, diarrhea and some general signs of intoxication
██████████, 2002*; ASB2012-11503; Feinchemie	Acute dermal toxicity Rat, CD	98.0	Limit test (m/f)	LD ₅₀ > 2000 mg/kg bw, no evidence of toxicity
██████████ 1993; TOX9552396; Cheminova	Acute dermal toxicity Rat, SD	99.2	Limit test (m/f)	LD ₅₀ > 2000 mg/kg bw, no evidence of toxicity
██████████ 1993; TOX9300374; Cheminova	Skin sensitisation Guinea pig (Female Dunkin-Hartley)	99.2	Maximisation test (M&K)	Not sensitising
██████████, 2002*; ASB2012-11506; Feinchemie	Skin sensitisation Guinea pig (Male Dunkin-Hartley)	98.0	Maximisation test (M&K)	Not sensitising
██████████ 1993; TOX9300349; Cheminova	4-week oral toxicity, Rat, SD	99.2	Gavage; 0, 10, 100, 350, 1000 mg/kg bw/d (m/f)	NOAEL = 100 mg/kg bw/day, based on higher kidney wt in m and lower bw gain in f
██████████ 1993; TOX9300377; Cheminova	13-week oral toxicity, Rat, SD	99.2	Gavage; 0, 10, 100, 1000 mg/kg bw/d (m/f)	NOAEL = 1000 mg/kg bw/day

Reference; study identification, owner	Type of study; Species, strain	AMPA purity [%]	Exposure conditions / test method; dose levels	Results
██████████ 1979; TOX9552401; Monsanto	90-day oral toxicity, Rat, CD	99.96	Feeding (adjusted for dose levels); 0, 400, 1200, 4800 mg/kg bw/d (m/f)	NOAEL = 400 mg/kg bw/day, based on bw gain↓, urothelial hyper-plasia (bladder) and gastro-intestinal clinical signs; at top dose level in addition mortality following blood collection (f), food consumption↓, clinical chemistry findings (LDH activity↑) and hyper-plasia of renal pelvis epithelium
██████████ 1991; TOX9552406; Monsanto	90-day oral toxicity, Beagle dog	87.8	Capsule; 8.8, 26.3, 87.8, 263 mg/kg bw/d when adjusted for purity (m/f)	NOAEL = 263 mg/kg bw/day; no treatment-related findings
██████████ 1993; TOX9300378; Cheminova	Genotoxicity in bacteria; <i>S. typhimurium</i> TA100, TA98, TA1535, and TA1537	99.2	Ames test, ±S9 mix for metabolic activation, 310-5000 µg/plate, plate incorporation and pre-incubation assay	Negative
██████████ 1993; TOX9300380; Cheminova	Genotoxicity in mammalian cells, Mouse lymphoma (L5178Y) cells	99.2	Mouse lymphoma assay, ±S9 mix for metabolic activation, 310-5000 µg/mL	Negative
Callander, 1988; TOX9500043; Syngenta	Genotoxicity in bacteria; <i>S. typhimurium</i> TA100, TA98, TA1535, TA1537, TA1538, and <i>E. coli</i> (WP2 <i>uvrA</i>)	> 99	Ames test, ±S9 mix for metabolic activation, 1.6 - 5000 µg/plate, plate incorporation and (only with S9 mix) pre-incubation assay	Negative
Akanuma, 1996*, ASB2012-11507; Arysta	Genotoxicity in bacteria; <i>S. typhimurium</i> TA100, TA98, TA1535, TA1537 and <i>E. coli</i> (WP2 <i>uvrA</i>)	99.33	Ames test, ±S9 mix for metabolic activation, 313 - 5000 µg/plate, pre-incubation assay	Negative (supplementary study)

Reference; study identification, owner		Type of study; Species, strain	AMPA purity [%]	Exposure conditions / test method; dose levels	Results
	Nesslany, 2002*; ASB2012-11508; Arysta	Genotoxicity <i>in vitro</i> (DNA damage and repair); primary rat hepatocytes	99.9	UDS in rat hepatocytes, 0.625-10 mM	Negative
	Bakke, 1991; TOX9552409; Monsanto	Genotoxicity <i>in vitro</i> (DNA damage and repair); primary rat hepatocytes	94.38	UDS in rat hepatocytes, 5 – 5000 µg/mL	Negative up to 2500 µg/mL, meaningful evaluation of higher concentrations not possible due to cytotoxicity
	██████, 1993; TOX9300379; Cheminova	Genotoxicity (clastogenicity) <i>in vivo</i> , Mouse, NMRI	99.2	Micronucleus test in bone marrow, 5000 mg/kg bw, single oral exposure (m/f)	Negative
	██████, 1993; TOX9552413; Monsanto	Genotoxicity (clastogenicity) <i>in vivo</i> , Mouse, CD-1	94.38	Micronucleus test in bone marrow, 100, 500, 1000 mg/kg bw, single i.p. injection (m/f)	Negative
	██████, 1992; TOX9300348; Cheminova	Developmental toxicity Rat, SD	99.2	0, 100, 350, 1000 mg/kg bw/d by gavage, gestation days 6-16	NOAEL = 1000 mg/kg bw/day (maternal and developmental)
	██████, 1991; TOX9552414; Monsanto	Developmental toxicity Rat, SD	94.38	0, 150, 400, 1000 mg/kg bw/d by gavage, gestation days 6-15	Maternal NOAEL 150 mg/kg bw/day, based on clinical signs, bw gain/food consumption↓; Developmental NOAEL 400 mg/kg bw/d, based on mean fetal wt↓

* Study previously not evaluated by the EU

Acute oral toxicity in the mouse (██████ 1996)

Reference:

IIA, 5.8/01

Report:

██████ 1996 AMPA: Acute Oral Toxicity Study In Mice.

██████, Data owner: Arysta Life Sciences

Report No.: IET 96-0075

Date: 1996-11-11

not published, ASB2012-11502

Guidelines:

OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)

Deviations:

None

GLP:

Yes

Acceptability: See RMS comment

Dates of experimental work: 1996-09-24 to 1996-10-08

Materials and methods

Test material:

Identification: AMPA

Description: White powder

Lot/Batch #: A-960719

Purity: 99.33 %

Stability of test compound: Stable for 1 year at RT.

Vehicle: 1 % carboxymethyl-cellulose (CMC)

Test animals:

Species: Mice

Strain: ICR (Crj:CD-1), SPF

Source:

Age: 6 weeks

Sex: Male and females

Weight at dosing: ♂ 30.5 – 34.6 g; ♀ 22.9 – 24.8 g

Acclimation period: 7 days

Diet/Food: Pellet Diet MF (Oriental Yeast Co., Japan), *ad libitum* except for approx. 3 h before and after dosing

Water: Tap water, *ad libitum*

Housing: Aluminium cages with wire-mesh floors in groups of 5 animals/sex/cage.

Environmental conditions: Temperature: $23 \pm 3^{\circ}\text{C}$

Humidity: $55 \pm 15\%$

Air changes: 12/hour

12-hour light/dark cycle

Animal assignment and treatment:

A group of five fasted mice per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made 1, 3 and 6 h after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anaesthesia and subjected to gross necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No clinical signs were observed during the study.

Body weight: No body weight losses were recorded in any animal 7 and 14 days after the administration.

Necropsy: The gross necropsy conducted at termination of the study noted no observable abnormalities.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (AMPA) was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute oral toxicity.

RMS comments:

The study is considered acceptable. The conclusion is agreed with.

Acute oral toxicity in the rat (1988)

Reference: IIA, 5.8/02

Report: 1988

Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat.

Data owner: Syngenta

Report No.: CTL/P/2266

Date: 1988-08-10

GLP:

not published, TOX9500044

Guidelines: Not stated, but method is in accordance with OECD 401.

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: No date given in the report.

Materials and methods

Test material:

Identification: Aminomethyl Phosphonic Acid (AMPA)

Description: White solid

Lot/Batch #: Y06384/001/001 (CTL reference)

Purity: 100 % (assumed)

Stability of test compound: No data given in the report.

Vehicle: 0.5 % (w/v) aqueous polysorbate 80

Test animals:

Species: Rat

Strain: Wistar (Alpk:APfSD), SPF

Source:

Age: Approx. 8-9 weeks

Sex: Male and females

Weight at dosing: ♂ 280 – 312 g; ♀ 204 – 214 g

Acclimation period: At least 6 days

Diet/Food:	Porton Combined Diet (Special Diets Services Ltd.), <i>ad libitum</i> except for approx. 24 h before dosing
Water:	Tap water, <i>ad libitum</i>
Housing:	Suspended stainless steel/polycarbonate cages with stainless steel mesh floors in groups of max. 5 animals/sex/cage.
Environmental conditions:	Temperature: 15 – 24 °C Humidity: 50 ± 10 % Air changes: 20 – 30/hour 12-hour light/dark cycle

Animal assignment and treatment:

A group of five fasted rats per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 10 mL/kg bw. One animal was accidentally killed (by mis-dosing) on Day 1 and another animal was therefore substituted, but was dosed one day later. Observations for mortality and clinical/behavioural signs of toxicity were made once 30-90 minutes, 4 and 6 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded one day prior to dosing, the day of dosing (Day 1) and on Days 3, 5 or 6, 8 and 15. On Day 15 after dosing, each animal was euthanized under ether anaesthesia and subjected to gross necropsy.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Signs of slight toxicity (diarrhoea, chromodacryorrhea, piloerection, stains around nose, ungroomed appearance, signs of urinary incontinence) were seen in the animals, but these did not persist and all animals had recovered by Day 3 or 4.

Body weight: All animals lost weight initially due to the pre-dose fast, but all then gained weight and had exceeded their initial bodyweight by Day 6. Moreover, one male lost weight between Day 6 and 8 and one further male and three females between Day 8 and 15. The reason was unclear as there were no associated clinical abnormalities, nor were there any abnormalities at necropsy.

Necropsy: The gross necropsy conducted at termination of the study noted no observable abnormalities.

Conclusion by the Notifiers

The oral LD₅₀ of the test material (AMPA) was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute oral toxicity.

RMS comments:

This study is considered acceptable and its conclusion is agreed with. It should be noted that it was already evaluated by the RMS (and later on by the EU) when submitted as part of the toxicological data package for glyphosate-trimesium (DAR, 1998, ASB2010-10493)

Acute dermal toxicity in the rat [REDACTED] 2002)**Reference:** IIA, 5.8/03**Report:** [REDACTED] 2002

Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration – LIMIT TEST

Data owner: Feinchemie Schwebda GmbH

Report No.: 16168/02

Date: 2002-12-03

not published, ASB2012-11503

Guidelines: OECD 402 (1987), EEC B.3 (1992)**Deviations:** None**GLP:** Yes**Acceptability:** See RMS comment

Dates of experimental work: 2002-10-21 to 2002-11-01

Materials and methods

Test material:

Identification: AMPA (Aminomethyl Phosphonic Acid)

Description: White solid powder

Lot/Batch #: FA005563

Purity: 98.0 %

Stability of test compound: Stable until 2004-12-03 at RT.

Vehicle: 0.5 % aqueous hydroxypropylmethyl cellulose gel

Test animals:

Species: Rat

Strain: CD / CrI:CD

Source: [REDACTED]

Age: 20 - 22 days

Sex: Male and females

Weight at dosing: ♂ 214 – 238 g; ♀ 213 – 223 g

Acclimation period: At least 5 days.

Diet/Food: ssniff R/M-H V1530 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* except for approx. 16 h before dosingWater: Tap water, *ad libitum*

Housing: Individually in MAKROLON cages (type III) with granulated textured wood as bedding.

Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 55 ± 15 %
Air changes: not reported
12-hour light/dark cycle

Animal assignment and treatment:

The acute dermal toxicity of AMPA was tested on five male and five female CD rats. One day before treatment the administration site was clipped free of hair. A single dose of 2000 mg/kg bw test substance prepared as suspension in 0.5 % aqueous hydroxypropylmethyl cellulose gel was applied uniformly over an area of about 10 % of the total body surface. The dosing volume was 10 mL/kg bw. The application site was covered with an occlusive dressing for 24 hours. After removal of the dressing, possible residual substance was removed. All animals were observed for overt signs of toxicity or behavioural changes before and immediately, 5, 15, 30 and 60 minutes, as well as 3, 6 and 24 h after administration and subsequently once daily for 14 days. Individual body weights were recorded before administration and on Days 7 and 14. All surviving animals were killed at the end of the 14-day observation period.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No clinical signs were observed during the study.

Body weight: No body weight losses were recorded in any animal 7 and 14 days after the administration.

Necropsy: The gross necropsy conducted at termination of the study noted no observable abnormalities.

Conclusion by the Notifiers

The dermal LD₅₀ of the test material (AMPA) was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute dermal toxicity.

RMS comments:

The study is considered acceptable and the conclusion is agreed with.

Skin sensitisation in the guinea pig by M&K test [REDACTED], 2002)

Reference:

IIA, 5.8/04

Report:

[REDACTED] 2002 Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitisation Test in Guinea Pigs according to Magnusson And Kligman (Maximisation Test)

[REDACTED]

Data owner: Feinchemie Schwebda GmbH

Report No.: 16169/02

Date: 2002-12-03

Unpublished, ASB2012-11506

Guidelines:

OECD 406 (1992); EEC B.6 (1996)

Deviations:

None

GLP:

Yes

Acceptability:

See RMS comment

Dates of experimental work: 2002-10-12 to 2002-11-26

Materials and methods

Test material:

Identification:	AMPA
Description:	White solid powder
Lot/Batch #:	FA005563
Purity:	98.0 %
Stability of test compound:	At room temperature stable until December 31, 2004.
Vehicle:	Purified water
Test animals:	
Species:	Guinea pig
Strain:	Dunkin Hartley
Source:	
Age:	22 days
Sex:	Male
Weight at dosing:	252 - 307 g; positive control group: 228 - 341 g
Acclimation period:	At least 5 days.
Diet/Food:	ssniff Ms-H (ssniff Spezialdiäten GmbH, Soest, Germany), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	In pairs in Makrolon cages (type IV) with granulated textured wood bedding.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: no data 12 hours light/dark cycle

Animal assignment and treatment:

AMPA was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Male Dunkin Hartley guinea pigs, young adults with body weights ranging from 228 to 341 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals. The main study was performed in 10 test animals, 5 control animals and 20 positive control animals.

The induction phase consisted of an intradermal injection at Day 0 and an epidermal application on Day 7. On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin of the shoulder region at a concentration of 5% either in purified water or in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and purified water.

On Day 6 the skin was shaved and coated with 0.5 mL sodium laurylsulfate 10% in vaseline in order to induce a local irritation. On Day 7 the test substance was topically applied at a concentration of 50 % to the clipped and shaved skin of the shoulder region and covered with an occlusive dressing, which was left in place for 48 hours.

The challenge was conducted on Day 21 by an occlusive patch at a concentration of 50% which was applied to the clipped and shaved left flank of each animal for 24 h. The clipped and shaved right flank of each animal was treated in the same way with the vehicle only. 24

and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale.

The animals of the positive control group were treated with a 2 % benzocaine solution intracutaneously in the induction phase and with a 5 % solution topically in the induction phase and at challenge.

Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period.

Evaluation criteria for classification as a potential skin sensitizer:

At the 24-hour and/or 48-hour reading, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

Results and discussion

Mortality: No deaths occurred.

Clinical observations: No signs of systemic toxicity were observed.

Body weight: All animals showed the expected gain in body weight.

Skin reactions: No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group.

Animals treated with the positive control benzocaine in 40% ethanolic 0.9% NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Conclusion by the Notifiers

Based on the study results and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for skin sensitization.

RMS comments:

The study is considered acceptable. AMPA was not a skin sensitiser in the M&K test.

1st “new” mutagenicity (Ames) test in bacteria (Callander, 1988)

Reference:	IIA 5.8/05
Report:	Callander, R.D. 1988 Aminomethyl Phosphonic Acid: An Evaluation of Mutagenic Potential Using <i>S. Typhimurium</i> and <i>E. Coli</i> ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK Data owner: Syngenta Report No.: CTL/P/2206 Date: 1988-09-21 not published, TOX9500043
Guidelines:	Comparable to OECD 471 (1997): OPPTS 870.5100 (1998): 2000/32/EEC B.13/B.14 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	See RMS comment

Dates of experimental work: 1988-03-01 to 1988-09-21

Materials and methods

Test Material: Aminomethyl phosphonic acid
 Description: Crop metabolite of, and an impurity in, ICIA0224, white solid
 Lot/Batch number: 48F-3893
 Purity: >99 % a.i.
 CAS#: Not reported
 Stability of test compound: Confirmed by Sponsor

Control Materials:

Negative: Water

Solvent control Dimethylsulphoxide – DMSO (10 µl/plate)

(final concentration):

Positive control: Nonactivation:

Acridine mutagen ICR191 TA1537

2-Aminoanthracene TA1537 WP2 uA

Daunomycin hydrochloride TA98

4-Nitro-o-phenylene diamine TA1538

N-Methyl-N'-nitro-N-nitrosoguanidine TA1535, TA100, WP2 uA

Activation:

2-Aminoanthracene TA1535, TA1537, TA1538, TA98, TA100, WP2 uA

Acridine mutagen ICR191 TA1537

2-Aminoanthracene TA1535, TA1537, TA1538, TA98, TA100, WP2 uA

N-Methyl-N'-nitro-N-nitrosoguanidine WP2 uA

Mammalian metabolic system: S9 derived

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other β-naphthoflavone		Other		

The metabolic activation system (S9-mix) used in this study was prepared as a 3:7:20 mixture of S9 fraction, Sucrose-tris-EDTA buffer (250:50:1 mM) and cofactor solution.

The cofactor solution was prepared in bulk as follows: Na₂HPO₄ (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and MgCl₂ (12 mM).

Test organisms:

<i>S. typhimurium</i> strains									
	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537	X	TA1538		list any others		
<i>E. coli</i> strains									
	WP2 (pKM101)	X	WP2 uvrA (pKM101)						
Properly maintained?							X	Yes	No
Checked for appropriate genetic markers (rfa mutation, R factor)?							X	Yes	No

Test compound concentrations used:

Nonactivated conditions: 5000, 1000, 200, 40, 8 and 1.6 µg/plate

Activated conditions: 5000, 1000, 200, 40, 8 and 1.6 µg/plate

For all strains triplicate plates were used for all test substance and positive control treatments.

For solvent controls 5 plates were used.

Study design and methods

Preliminary Cytotoxicity Assay: Not performed.

Type of Bacterial assay:

X standard plate test (both experiments –S9, initial experiment +S9)

___ pre-incubation (60 minutes) (second experiment +S9)

___ “Prival” modification (i.e. azo-reduction method)

___ spot test

___ other

Protocol:

Bacterial cultures were prepared from frozen stocks by incubating for 10-12 hours at 37 °C.

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µL Test solution at each dose level, solvent and positive controls;

500 µL S9 mix or phosphate buffer;

100 µL Bacteria suspension;

2 mL Overlay agar containing 50 µM histidine or tryptophan as appropriate.

In this assay 100 µL aliquots of an overnight culture of each bacteria strain were stored in bijou bottles at room temperature until required (1-2 hours). 500 µL S9 mix (or Co-factor/Buffer mix) was then added by dispensing syringe to the number of bijou bottles of one strain required for one dose level, followed by 0.1 mL of the appropriate concentration of the test substance solution added by micropipette. Finally, 2.0 mL top agar was added to each bijou, the force of addition was sufficient to mix the contents. The mixture was then rapidly poured onto a prepared Vogel Banner agar plate. After the agar was set the plates were incubated upside down for 64 - 68 hours at 37 °C in the dark. For each strain and dose level including the controls, three plates were used.

Following the total incubation period the plates were examined for the lack of microbial contamination and evidence that the test was valid: i.e. there should be a background lawn on the negative control plates and on the plates for (at least) the lower doses of test substance, and that the positive controls should show at least a two-fold increase in average reversion frequency rate and there should be a dose-response relationship.

The plates were counted using an automated colony counter (AMS 40-10) with the discrimination adjusted appropriately to permit the optimal counting of mutant colonies.

Statistical analysis: None – see Evaluation Criteria below.

Evaluation criteria: A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met:

a significant, dose-related increase in the mean number of revertants is observed;

a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) is observed at one or more concentrations

A negative result in a (valid) individual experiment is achieved when:

there is no significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance; and

in the absence of any such dose response, no increase in colony numbers is observed (at any test concentration) which exceeds 2x the concurrent solvent control.

For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain/S9 combination, then the observed effect(s) must be consistently reproducible.

Results

Mutagenicity assay: In two separate experiments, aminomethyl phosphonic acid did not induce any significant increases in the observed numbers of revertant colonies in *Salmonella typhimurium* strains TA1535, TA1538, TA98, TA100 and *Escherichia coli* WP2 *uvrA* pKM101 in either the presence or absence of an auxiliary metabolising system (S9).

In the first experiment, slight responses were observed in strain TA1537, reaching maxima of 1.9 x and 2.0 x background in the presence and absence of S9 respectively. These responses were only of limited dose-relationships, and were of limited statistical significance in both cases. In two further experiments, no significant increases in colony numbers were observed either with or without S9. This lack of reproducibility indicates that the observed effects in the first experiment are not due to compound-induced mutations.

The positive controls for each experiment induced the expected responses indicating the strains were working satisfactorily in each case.

Conclusion by the Notifiers

Under the conditions of this assay, aminomethyl phosphonic acid gave an unequivocal negative, ie non-mutagenic, response, when tested to a limit dose of 5000 µg/plate.

RMS comments

The study is considered acceptable. No evidence of genotoxicity of AMPA in bacteria was obtained. The slight increase in revertant numbers in one strain in the first experiment was rather weak and was sufficiently contravened by subsequent trials in which the test material proved clearly negative.

It should be noted that this study was already evaluated by the RMS (and later on by the EU) when submitted as part of the toxicological data package for glyphosate-trimesium (DAR, 1998, ASB2010-10493)

2nd new mutagenicity (Ames) test in bacteria (Akanuma, 1996)

Reference: IIA, 5.8/06
Report: Akanuma M. 1996 AMPA Reverse mutation test.
 The Institute of Environmental Toxicology, Tokyo, Japan
 Data owner: Arysta LifeScience
 Study No.: IET 96-0076
 Date: 1996-12-09
 not published, ASB2012-11507
Guidelines: U.S. EPA FIFRA Guidelines, Subdivision F
 OECD guidelines 471, 472 (1983)
 Japan MAFF guidelines 59 NohSan N° 4200 (1985)
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1996-09-09 to 1996-10-11

Materials and methods

Test material: AMPA
 Identification: AMPA
 Description: White powder
 Lot/Batch #: A-960719
 Purity: 99.33 %
 Stability of test compound: Stable for 1 year at room temperature
 Solvent used: Sterile water
 2. control materials:
 Negative: Sterile water
 Solvent/final concentration: Water / 50 mg/mL
 Positive: non-activation
 and activation

Strain	Positive controls	
	Without S9 (µg/plate)	With S9 (µg/plate)
TA100	AF-2 (0.01)	2-AA (1)
TA1535	NaN ₃ (0.5)	2-AA (2)
WP2 uvra	AF-2 (0.01)	2-AA (10)
TA98	AF-2 (0.1)	2-AA (0.5)
TA1537	9-AA (80)	2-AA (2)

AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide dissolved in DMSO; NaN₃ : sodium azide dissolved in sterile water

2-AA: 2-aminoanthracene dissolved in DMSO; 9-AA: 9-aminoacridine hydrochloride dissolved in sterile water

activation: The enzyme activity measured by mutagenicity was good. S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl₂, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.

test organisms: *Escherichia coli* WP2 uvrA
Salmonella typhimurium (TA100, TA1535, TA98 and TA1537)

test concentrations:

Preliminary cytotoxicity assay: One preliminary assay was performed:

Plate incorporation assay: Concentrations up to 5000 µg/plate were evaluated with and without S9 activation in strain TA1535, TA1537, TA98, TA100 and WP2 uvrA. A single plate was used, per dose, per condition.

Pre-incubation assay: As above.

Mutation assays:

Plate incorporation assay: 156, 313, 625, 1250, 2500 and 5000 µg/plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.

Pre-incubation assay: As above for the plate incorporation assay.

The independently repeated mutation assay was conducted using the pre-incubation modification to the standard plate incorporation test. The pre-incubation assay was carried out as described above with the following two exceptions: 0.5 mL of buffer were added to cultures prepared for testing under non-activated conditions; prior to the addition of top agar, reaction mixtures were incubated for 20 minutes at 37 ± 1°C.

Statistics

Results were judged without statistical analysis.

Evaluation Criteria

The test items were carried out twice. Reproducibility of results was confirmed by two independent experiments. Results were judged positive without statistical analysis when the following criteria are all satisfied:

A two-fold or greater increase above solvent control in the mean number of revertants is observed

This increase in the number of revertants is accompanied by a dose-response relationship

This increase in the number of revertants is reproducible.

Results and discussion

Analytical determinations: None

		Concentration	Revertants (n° colonies/plate)				
		(µg/plate)	TA100	TA1535	WP2 <i>uvr A</i>	TA98	TA1537
- S9 mix		Solvent Control (H ₂ O)	150*	8*	20*	24*	7*
		200	131	11	21	12	8
		500	152	10	19	22	6
		1000	131	10	15	20	5
		2000	154	3	23	24	10
		5000	140	11	20	18	11
+ S9 mix		Solvent Control (H ₂ O)	106*	6*	28*	25*	10*
		200	92	7	19	16	16
		500	116	1	20	24	15
		1000	124	5	25	17	12
		2000	102	7	24	30	8
		5000	128	10	29	26	11
Positive Control	- S9 Mix	Compound	AF-2	NaN ₃	AF-2	AF-2	9-AA
		µg/plate	0.01	0.5	0.01	0.5	80
		Revertants/plate	666*	673*	201*	658*	540*
	+ S9 Mix	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
		µg/plate	1	2	10	0.5	2
		Revertants/plate	450*	233*	415*	456*	84*

Mutation assays: Results are shown in the following tables.

	Concentration	Revertants (n° colonies/plate) *				
	(µg/plate)	TA100	TA1535	WP2 <i>uvr A</i>	TA98	TA1537
- S9 mix	Solvent Control (H ₂ O)	101±17	11±1	17±4	18±3	4±3
	313	84±7	10±5	17±6	14±2	5±3
	625	91±14	8±6	17±7	16±4	4±2
	1250	97±17	8±3	14±2	13±2	4±2
	2500	91±7	9±1	15±8	15±2	6±2
	5000	100±4	7±3	16±4	16±5	3±1
Positive Control (- S9)	Compound	AF-2	NaN ₃	AF-2	AF-2	9-AA
	µg/plate	0.01	0.5	0.01	0.1	80
	Revertants/plate	619±57	619±45	160±22	667±60	710±73

* : Average \pm SD

Table B.6.8-4: Reverse mutation tests with metabolic activation– Experiment 1

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 <i>uvr A</i>	TA98	TA1537
+ S9 mix	Solvent Control (H ₂ O)	105±11	10±4	19±1	30±5	10±1
	313	105±5	12±2	16±4	28±5	9±3
	625	92±6	6±1	16±1	28±7	13±2
	1250	90±3	6±1	16±2	25±7	11±3
	2500	83±9	9±4	20±4	25±8	10±3
	5000	93±10	10±4	24±6	32±10	7±1
Positive Control (+S9)	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
	µg/plate	1	2	10	0.5	2
	Revertants/plate	529±33	184±5	384±20	407±11	94±2

*: Average ± SD

Table B.6.8-5: Reverse mutation tests without metabolic activation– Experiment 2

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 <i>uvr A</i>	TA98	TA1537
- S9 mix	Solvent Control (H ₂ O)	120±3	9±3	15±3	18±4	3±2
	313	136±9	4±1	18±3	14±4	4±3
	625	124±16	5±2	16±3	13±3	3±2
	1250	107±11	6±4	12±4	15±2	3±3
	2500	96±6	9±4	12±3	16±6	4±0
	5000	117±2	7±3	20±5	13±2	3±2
Positive Control (-S9)	Compound	AF-2	NaN ₃	AF-2	AF-2	9-AA
	µg/plate	0.01	0.5	0.01	0.1	80
	Revertants/plate	668±27	696±20	182±16	650±8	698±53

*: Average ± SD

Table B.6.8-6: Reverse mutation tests with metabolic activation– Experiment 2

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 <i>uvr A</i>	TA98	TA1537
+ S9 mix	Solvent Control (H ₂ O)	95±3	8±2	17±3	28±5	7±2
	313	112±14	8±3	17±4	21±6	10±5
	625	84±5	7±4	16±5	21±5	7±3
	1250	106±8	7±2	17±4	28±9	7±1
	2500	97±4	11±3	16±2	21±1	6±1
	5000	115±12	9±5	22±2	22±3	6±5
Positive Control (+S9)	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
	µg/plate	1	2	10	0.5	2
	Revertants/plate	584±56	169±28	461±8	334±14	82±4

*: Average ± SD

Conclusion by the Notifiers

A two-fold or greater increase in the mean number of revertant colonies was not observed in any strain at any dose of AMPA in the reverse mutation tests with or without metabolic activation. It is concluded that AMPA is non mutagenic for bacteria under the conditions used with this experiment.

RMS comments:

In contrast to the description in the dossier, it seems that testing was performed by means of the pre-incubation method only. Therefore, the study is considered supplementary only because a plate-incorporation test was not performed.

When the study description in the dossier was compared to the original study report, it was noted that the study director was Mie Akanuma. Erroneously, the first name had been mentioned in the dossier instead of the authors surname.

UDS assay for DNA damage and repair *in vitro* (Nesslany, 2002)

Reference: IIA, 5.8/07
Report: Nesslany, F. 2002 Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* procedure with AMPA (Amino methyl phosphonic acid).
 The Institute of Environmental Toxicology, Tokyo, Japan
 Data owner: Arysta LifeScience
 Study No.: IPL-R-02025
 Date: 2002-09-10
 not published, ASB2012-11508
Guidelines: OECD guideline n° 482
Deviations: None
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 2002-04-29 to 2002-07-02

Materials and methods

Test material: AMPA
 Identification: AMPA
 Description: White crystalline powder
 Lot/Batch #: 020404
 Purity: 99.9 %
 Stability of test compound: Not mentioned in the report
 Solvent used: William's E medium Gibco
 2. control materials:
 Negative: /
 Solvent/final concentration: See above
 Positive: 2-acetamidofluorene
 activation: None
 test organisms: Rats hepatocytes
 test concentrations: 5 dose level were tested: 10, 5, 2.5, 1.25 and 0.625 mM with and without S9 metabolic activation

Test principle

Hepatocytes were isolated from livers of rats. The primary hepatocyte cultures were exposed to the test article in the presence of ³H thymidine which is incorporated into the DNA if DNA damage is occurring. DNA repair systems then stimulated UDS and increased the incorporation of thymidine which was measured by grain counting after autoradiography of hepatocytes.

The following results are presented:

The average NNG and standard deviation

The percent of cells in repair and standard deviation (≥ 5)

The average cytoplasmic and nuclear grain count

The number of cells in S-phase

Statistics

Results were judged without statistical analysis.

Evaluation Criteria

Results are judged positive when:

At any dose tested, group, mean NNG value greater than 0 NNG and 20 % or more of cells responding (NNG value ≥ 5)

An increase is seen in both NNG and the percentage of the cells in repair

A dose related increase is seen in both NNG and the percentage of the cells in repair

Any induction of UDS can be reproduced in an independent experiment.

Validity Criteria

The assay is considered valid if:

Negative control slides have a group mean NNG value within the historical range.

The positive control have group mean NNG values of less than 5 NNG counts with 50% or more cells having NNG counts of 5 or more and statistically significant relative to the solvent control.

Results and discussion

Analytical determinations: None

Mutation assays: Negative control gave a group mean NNG value of less than zero with a percentage of cells in repair comparable with historical control data. In positive controls, group mean net nuclear grain count (NNG) values as well as percentage of cells in repair obtained were within the range of historical control. The sensitivity of the cell type used to a DNA damaging agent requiring metabolism for its action, 2-acetamidofluorene was demonstrated. Thus, the validity criteria of the test were fulfilled.

The findings of both experiments are summarised in the tables below.

Table B.6.8-7: UDS data of the first experiment

Concentration (mM)	Net Nuclear Grain Count (NNG)		Net Nuclear Grain Count of cells in repair (NNG >5)		% cells in repair (NNG >5)	
	Mean	+/- sd	Mean	+/- sd	Mean	+/- sd
Solvent control	-2.38	5.20	5.63	0.51	4.21	1.53
0.625	-3.81	5.29	6.61	1.56	3.89	1.53
1.25	-3.61	5.23	6.99	0.56	4.02	1.53
2.5	-3.93	5.24	6.83	0.91	5.18	1.53
5	-3.35	5.10	6.28	1.66	3.45	1.00
10	-2.04	4.82	6.91	0.74	5.43	1.53
Positive control: 2-acetamidofluorene 6.25 µM	30.81	18.22	31.55	4.84	96.61	2.00

Table B.6.8-8: UDS data of the second experiment (*amended by RMS because of errors*)

Concentration (mM)	Net Nuclear Grain Count NNG		Net Nuclear Grain Count of cells in repair NNG >5		% cells in repair NNG >5	
	Mean	+/- sd	Mean	+/- sd	Mean	+/- sd
Solvent control	-4.62	5.81	5.37	0.21	3.74	2.08
0.625	-4.77	5.46	6.90	0.60	1.78	0.00
1.25	-4.04	5.35	6.87	1.00	4.49	1.53
2.5	-3.81	5.82	7.07	1.61	6.18	2.08
5	-3.47	5.83	7.83	0.29	7.73	1.73
10	-5.58	6.06	6.97	1.29	3.76	1.53
Positive control: 2-acetamidofluorene 6.25 µM	17.57	11.18	17.73	3.29	94.94	9.45

Over the two experiments, group mean net nuclear grain count (NNG) values at the dose range tested from 10 to 0.625 mM were less than zero (-2.04 to -3.81 vs -2.38 in solvent control in the first UDS assay and -5.58 to -4.77 vs -4.62 in solvent control in the second UDS assay), that is to say below the threshold value of 0 NNG for a positive response.

Furthermore, no significant increase in the percentage of cells in repair at any dose of AMPA tested when compared with the respective controls (5.43 to 3.89% vs. 4.21% in solvent control in the first assay and 3.76 to 1.78 % vs. 3.74% in solvent control in the second assay). In addition, in cells in repair, group mean net nuclear grain count (NNG≥5) values were comparable with the solvent controls (6.91 at 10 mM to 6.61 at 0.625 mM vs. 5.63 in control in the first assay and 6.97 to 10 mM to 6.90 at 0.625 mM vs. 5.37 in control in the second assay).

Conclusion by the Notifiers

Under the conditions of this experiment, AMPA did not reveal any genotoxicity activity in the Unscheduled DNA synthesis assay.

RMS comments:

The study is considered acceptable. There was no evidence of a direct interaction of AMPA with the DNA of primary rat hepatocytes.

In contrast to what was mentioned in the GTF dossier, the test assay was performed in the “Institut Pasteur de Lille” in France and not in Japan.

B.6.8.2 Mechanistic studies on certain aspects of the toxicity of glyphosate**B.6.8.2.1 Studies on mechanism of salivary gland findings**Introduction by RMS:

A few studies have been submitted to investigate the mechanism behind the salivary gland findings that were obtained in quite many subchronic, long-term and reproduction studies in rats (see sections B.6.3, B.6.5 and B.6.6) and occasionally also in mice (██████████, 1992, TOX9551954). The first experiment to elucidate the mode of action was performed by ██████████ (1992, TOX9551954) as part of their comprehensive investigations within the U.S. NTP and is described in the original DAR (1998, ASB2010-10302) on glyphosate in detail. The study was considered acceptable upon re-evaluation. In order to provide a most comprehensive evaluation of salivary gland changes, this part from the old DAR is copied here, followed by a summary Table B.6.8-9 and the detailed description of two more recent studies by ██████████ (1996, ASB2012-11537/ASB2012-11520) and ██████████ (2010, ASB2012-11519), commented by the RMS.

Copy from the previous DAR: Possible explanation for salivary gland alterations

“Because of morphologic similarities to salivary gland changes observed with the adrenergic agonist isoproterenol, a subacute study was designed to test the hypothesis that the salivary gland effects of glyphosate were also mediated through an adrenergic mechanism (██████████, 1992, TOX9551954). Groups of four male F344/N rats received glyphosate at a dietary level of 50000 ppm or were fed an untreated control diet. In three of the groups, the adrenergic agonist isoproterenol and/or the antagonist propranolol were administered by continuous subcutaneous infusion by osmotic minipumps. The study design was as follows:

Group 1: control diet, only vehicle (water + 0.1% ascorbate) administered by minipump;

Group 2: glyphosate diet, only vehicle administered by minipump;

Group 3: glyphosate diet, propranolol (1.2 mg/kg bw/d) administered by minipump;

Group 4: control diet, isoproterenol (1.0 mg/kg bw/d) administered by minipump;

Group 5: control diet, isoproterenol and propranolol administered by minipump.

After 14 days of treatment, the left parotid and submandibular/sublingual salivary glands were removed, weighed separately and processed for electron microscopy. The right parotid and submandibular/sublingual salivary glands were removed, sectioned and stained for histological evaluation.

Both isoproterenol and glyphosate induced significant enlargement of the salivary glands, glyphosate having much greater effect than isoproterenol. The parotid was most affected. Propranolol inhibited the effect of both substances on salivary gland weight but not completely in the case of glyphosate. Microscopically, similar changes were induced by glyphosate and isoproterenol consisting of cytoplasmic basophilic change, fine vacuolation and swelling of acinar cells resulting in a relative reduction in the number of ducts present. Glyphosate-treated animals were most severely affected. Propranolol, however, clearly protected the rats from the more severe lesions. Likewise, modest protection of histological

effects caused by isoproterenol was seen. Cytoplasmic alteration of the submandibular gland was more subtle and histologically detectable only in glyphosate-treated animals. However, electron microscopy elucidated an effect of isoproterenol on this gland, too. It could not be determined if the serous or mucous glandular acini were selectively affected by glyphosate. No changes were seen in the sublingual glands examined from any group demonstrating target specificity of glyphosate- and isoproterenol-associated lesions to those salivary glands which are mainly innervated by adrenergic fibers. The authors assume that effects of glyphosate on salivary glands were due to an adrenergic mechanism. The biological significance of this finding is unknown ([REDACTED] 1992, TOX9551954).”

Table B.6.8-9: Summary of new mechanistic studies on salivary gland effects

Reference; study identification; owner		Type of study; species, strain	Application route (dose)	Test substance	Purity [%]	Results
Studies not reviewed in the 2001 evaluation	2010; ASB2012-11519; Monsanto	8-week oral toxicity; Sprague-Dawley Rat, ♂	Citric acid: Oral gavage (791-1316 mg/kg bw) or via diet (14000 ppm); Trisodium citrate dehydrate: diet (21400 ppm)	Citric acid, Trisodium citrate dihydrate	99.3	Higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands
	1996; ASB2012-11520 and 2012-115337; Syngenta	4-week oral toxicity; Sprague-Dawley (CD)/Fischer 344/Alpk:APSD (AP), Rat, ♂	Diet (20000 ppm)	Glypho-sate acid, batch P24	95.6	Marked strain differences in the severity of effect in the parotid salivary glands; most pronounced effect occurred in the F344 strain: diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells; similar but slighter an apparently reversible effects occurred in the AP and CD strains involving small foci of cells only

Effects of citric acid ([REDACTED] 2010)

Reference: IIA, 5.10/01

Report: [REDACTED] (2010)

An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats

Data owner: Monsanto/GTF

Study No.: WIL-50361

Date: 2010-01-08

Unpublished

ASB2012-11519

Guidelines:

Guideline does not exist for this kind of study but data from the

study report is similar to OECD 408.
Deviations: not applicable
GLP: yes
Acceptability: *See RMS comment*

Dates of experimental work: 2009-02-24 to 2009-05-15

Materials and methods

Test materials:

Identification: Anhydrous Citric Acid
Description: White powder
Lot/Batch #: XR3050
Purity: 99.9 %
Stability of test compound: Stable at room temperature until 2010-01-06.

Identification: Trisodium Citrate Dihydrate (TCD)
Description: White crystalline solid
Lot/Batch #: 1387609
Purity: 99.3 %
Stability of test compound: Stable at room temperature until 2011-03-01.
Vehicle: Gavage: deionised water, Diet: plain diet

Test animals:

Species: Rats
Strain: Sprague-Dawley (CD)

Source:

Age: prox. 6 weeks upon beginning of treatment

Sex: Males

Weight at dosing: 177 - 227 g

Acclimation period: 14 days

Diet/Food: Certified Rodent LabDiet #5002 (PMI Nutrition International, LLC), *ad libitum*

Water: tap water, *ad libitum*

Housing: Upon arrival, animals were housed three per cage for approximately 3 days. Thereafter, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board.

Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 50 ± 20 %
Air changes: at least 10/hour
12 hours light/dark cycle

Animal assignment and treatment:

In a 8 week gavage and feeding study, groups of 10 Sprague Dawley rats received the respective vehicles or test substances for 56 consecutive days via oral gavage (Groups 1 and 3) or in the diet (Groups 2, 4 and 5; see Table B.6.8-10). A low pH diet containing 14000 ppm of citric acid in basal diet was offered continuously to Group 4. A high pH diet containing 21400 ppm of trisodium citrate dihydrate in basal diet (at an equivalent citrate ion concentration to Group 4) was offered continuously to Group 5. A concurrent control group (Group 2) received the basal diet on a comparable regimen. Citric acid in the vehicle, deionised water, was administered orally by gavage at a dose level of 791-1316 mg/kg/day to Group 3.

Concentrations of the Group 3 formulations were calculated and adjusted weekly, based on the average food consumption and body weights of the Group 4 animals from the previous week of dosing in order to maintain approximately equivalent citric acid dose levels to Group 4. A concurrent gavage control group (Group 1) received the vehicle on a comparable regimen.

Table B.6.8-10: Study group assignment

Group Number	Test Substance application	Dose Level	Dose Volume	Number of animals
		(mg/kg bw/day or ppm)	(mL/kg)	
1	Gavage Vehicle	0	10	10
2	Basal Diet	0	na	10
3	Gavage Citric Acid (low pH)	791-1316	10	10
4	Diet Citric Acid (low pH)	14,000	na	10
5	Diet Trisodium Citrate (high pH)	21,400	na	10

na - not applicable

Observations

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly.

Body weight

Individual body weights were recorded weekly.

Food consumption and compound intake

Food consumption was recorded weekly.

Sacrifice and pathology

All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: parotid salivary glands, mandibular salivary glands and sublingual salivary glands. The mandibular and sublingual salivary glands were weighed together as one organ since they were fused and could not be adequately separated for weighing.

Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum and femur (incl. joint)), brain (cerebrum at two levels; cerebellum with medulla/pons), caecum, colon, duodenum, epididymides, eyes with optic nerves, gross lesions, harderian glands, heart, ileum, jejunum, kidneys, lacrimal gland (exorbital), liver, lungs (incl. bronchi), mammary gland, lymph nodes (mandibular, mesenteric and axillary), nasal cavity, oesophagus, pancreas, Peyer's patches, pituitary, prostate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerve, seminal

vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea and urinary bladder.

Microscopic examination was performed on the parotid salivary glands and gross lesions from all animals at the scheduled necropsy.

Statistics

All statistical tests were performed using the WIL Toxicology Data Management System (WTDMS™). Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1 % and 5 %, comparing each test substance-treated group to its respective control group.

Body weight, body weight change, food consumption, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA identified statistically significant ($p < 0.05$) intergroup variance, Dunnett's test was used to compare each of the test substance-treated groups to the respective control group (Group 1 to Group 3 and Group 2 to Groups 4 and 5). Group 1 was also compared to Group 2. Statistical analysis of the severity of histological changes was conducted. Individual animals were assigned severity scores based on parotid salivary gland changes (0=without histological change, 1=minimal change, 2=mild change, and 3=moderate change). The severity scores were then compared statistically using the Mann-Whitney U-test by comparing Group 1 to Group 3 and Group 2 to Groups 4 and 5.

Results and discussion

Mortality:

No deaths occurred during the study.

Clinical observations:

All clinical findings in the test substance-treated groups were noted with similar incidence in the control groups, were limited to single animals, and/or were common findings for laboratory rats of this age and strain.

Body weight:

There were no statistically significant differences when the respective control and test substance-treated groups were compared.

Food consumption:

Food consumption was unaffected by citric acid or trisodium citrate dihydrate administration. A statistically significant decrease in food consumption of the gavage citric acid group (Group 3, Week 7/8) was probably due to biological variability and not considered related to test substance administration.

Organ weights:

Test substance-related effects on organ weights consisted of statistically significant higher absolute and relative parotid salivary gland weights in the low pH diet group (14,000 ppm citric acid) when compared to the dietary control group; the magnitude of change was $> 40\%$ (Table B.6.8-11). Higher absolute and relative parotid salivary gland weights were also observed in the low pH gavage group (791-1316 mg/kg bw/day citric acid) and in the high pH diet group (21,400 ppm TCD) when compared to their respective control groups. However, the parotid salivary gland weight differences in the low pH gavage and high pH diet groups were not statistically significant and were of much lesser magnitude of change.

There were no other statistically significant test substance-related effects on the fused mandibular/ sublingual or parotid salivary gland weights when the control groups and test substance-treated groups were compared.

Table B.6.8-11: Organ weight differences of salivary glands

	Gavage Administration		Dietary Administration		
	aqueous control	791-1316 mg/kg bw/day citric acid	basal diet control	low pH diet, 14000 ppm citric acid	high pH diet, 21400 ppm trisodium citrate dihydrate
Mean Absolute Mandibular / Sublingual Fused Glands Weight (g)	0.7625 ± 0.05446	0.7873 ± 0.08397	0.7682 ± 0.08670	0.8872 ± 0.16548	0.7869 ± 0.07028
Mean Relative Mandibular / Sublingual Fused Glands Weight (g)	0.179 ± 0.0105	0.180 ± 0.0178	0.173 ± 0.0221	0.199 ± 0.0339	0.183 ± 0.0201
Mean Absolute Parotid Gland Weight (g)	0.3500 ± 0.12450	0.4082 ± 0.11990	0.2758 ± 0.08514	0.3905* ± 0.10920	0.3502 ± 0.08986
Mean Relative Parotid Gland Weight (g)	0.083 ± 0.0299	0.095 ± 0.0304	0.062 ± 0.0194	0.088* ± 0.0236	0.082 ± 0.0220

* - significantly different from relevant control group ($p < 0.05$) using Dunnett's test

Necropsy:

All macroscopic findings noted were considered spontaneous and/or incidental in nature and unrelated to test substance administration.

Histopathology:

Test substance-related histological effects consisted of a higher severity of cytoplasmic alterations in the parotid salivary glands of the citric acid and trisodium citrate dihydrate-treated groups when compared to their respective control groups (Table B.6.8-12). The severity of cytoplasmic alteration was increased in all dose groups; however, the cytoplasmic alteration was clearly most severe in the low pH diet group (Group 4; 14000 ppm citric acid). Cytoplasmic alteration in the parotid salivary glands was characterized by the presence of hypertrophied acinar cells with basophilic granular cytoplasm. The severity grades ranged from minimal to moderate, displayed by increasing numbers of affected acinar cells and more pronounced hypertrophy of acinar cells with increasing severity grade.

Cytotoxicity and hyperplasia were not observed and consequently, the observed changes were considered to be adaptive responses rather than adverse effects. There were no other test substance-related histological changes.

Table B.6.8-12: Toxicologically relevant histological changes

	Gavage Administration		Dietary Administration		
	aqueous control	791-1316 mg/kg bw/day citric acid	basal diet control	low pH diet, 14,000 ppm citric acid	high pH diet, 21,400 ppm trisodium citrate dihydrate
Parotid salivary glands ^a	9	10	10	10	10
Incidence (%)	100	100	70	100	90
Minimal	8	6	5	0	4
Mild	1	3	2	6	5
Moderate	0	1	0	4	0
Average severity ^b	1.1	1.5	0.9	2.4**	1.4

^a - number of tissues examined from each group

^b - 1= minimal, 2= mild and 3= moderate; animals without a histological change were assigned a severity score of 0

** - significantly different from relevant control group ($p < 0.01$) using the Mann-Whitney U-Test

Conclusion by the Notifiers

Citric acid administered orally via gavage or diet and trisodium citrate dihydrate administered via the diet to Sprague Dawley rats for 56 days resulted in higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands at all dose levels (791-1316 mg/kg bw/day gavage citric acid, 14000 ppm diet citric acid, and 21400 ppm diet trisodium citrate dihydrate). This effects were noted as most severe in the low pH dietary test group. In the absence of cytotoxicity and hyperplasia the noted effects are considered an adaptive response rather than an adverse effect and are consistent with the hypothesis that low pH diets result in adaptive cellular responses within the salivary glands.

RMS comments:

The study is considered supplementary because the test substance to be evaluated, i.e., glyphosate, was not included itself. However, it is suitable to demonstrate that organ weight and histological effects on salivary glands resembling very much those which were observed in some studies following glyphosate administration may be in fact due to low pH conditions in the oral cavity. Such conditions may occur when an acidic diet is administered as it was the case in feeding studies with glyphosate. It is agreed that the resulting salivary gland effects would be then rather adaptive than toxic. However, in principle, organ weight and histological changes may become adverse even if they are adaptive by nature. Furthermore, it cannot be excluded that other mechanisms might have also contributed to the observed findings in the glyphosate studies.

Comparison of sensitivity of different rat strains to salivary gland lesions (Allen, 1996)**Reference:** IIA, 5.10/02**Report:** [REDACTED] (1996)

Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat.

Data owner: Syngenta

Study No.: [REDACTED]/P/5160

Date: 1996-08-19

Unpublished

ASB2012-11520/11537

Guidelines: Guideline does not exist for this kind of study**Deviations:** not applicable**GLP:** yes**Acceptability:** See RMS comment

Dates of experimental work: 1996-01-15 to 1996-05-14

Materials and methods

Test materials:

Identification: Glyphosate acid

Description: White solid

Lot/Batch #: P24

Purity: 95.6 %

Stability of test compound: No data given in the report.

Vehicle and/
or positive control: Plain diet

Test animals:

Species: Rats

Strain 1: Alpk:AP_fSD

Source: [REDACTED]

Weight at dosing: 175.0 – 176.1 g

Strain 2: Sprague-Dawley

Source: Charles River UK Ltd., Margate, Kent, UK

Weight at dosing: 179.6 – 181.5 g

Strain 3: Fischer 344

Source: Harlan UK, Bicester, Oxon UK

Weight at dosing: 107.4 – 108.9 g

Age: approx. 28-30 days (on delivery)

Sex: Males

Acclimation period: 11-13 days

Diet/Food: CT1, *ad libitum*

Water: Tap water, *ad libitum*

Housing: Animals were housed by strain and four per cage.

Environmental conditions: Temperature: 21 ± 3 °C
Humidity: 50 ± 20 %
Air changes: at least 15/hour
12 hours light/dark cycle

Animal assignment and treatment:

In a 28 days feeding study groups of 24 male Alpk:AP_fSD (Wistar-derived: AP), Sprague-Dawley (Charles River CD; CD) and Fischer 344 (F344) rats received 0 or 20000 ppm glyphosate acid. Eight animals from each group were killed on Day 29 and the remaining animals were retained without treatment for a further 4 (8 rats/group) or 13 weeks (8 rats/group).

Two test diet batches were prepared prior to start of treatment by mixing 1255 g test substance to 58.745 kg diet and blending. Samples of both preparations were analysed to verify the achieved concentration.

Clinical observations

Clinical examinations were performed daily. A detailed physical examination was performed prior to administration and weekly thereafter.

Body weight

Individual body weights were recorded on start of administration and weekly thereafter.

Food consumption

Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage.

Sacrifice and pathology

All animals sacrificed at scheduled termination were subjected to a gross pathological examination of the salivary glands. Thereafter the salivary glands were removed, weighed (left and right separately) and examined by light microscopy.

Statistics

All data were evaluated using analysis of variance and/or covariance by the GLM procedure in SAS (1989). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based in the error mean square in the analysis.

Results and discussion

Analysis of dose formulations

The mean achieved concentration of glyphosate acid in both batches of diet was within 2% of the target concentration.

Mortality

There were no treatment-related deaths. One treated AP rat was killed in Week 7 following accidental damage to its snout.

Clinical observations

There were no treatment-related findings in any of the groups noted during the study period.

Body weight

AP rats: During the administration period significant reductions in group mean bodyweight compared to control were seen. At the end of the administration period the difference was approximately 7 %. The reduction in bodyweight was maintained during the 4-week recovery period (approximately 7 % at the end of Week 9) but no differences in body weight were apparent by the end of the 13-week recovery period.

CD rats: Group mean bodyweights for treated animals were significantly reduced during the administration period in comparison to controls. The reduction in body weight was approximately 7 % (after adjusting for initial body weight) at the end of the administration period. However, body weights quickly recovered and were 5 % higher than controls (after adjusting for initial body weight) by the end of the 13-week recovery period.

F344 rats: No treatment related effects were observed.

Food consumption

AP rats: Overall, food consumption in the treated group tended to be slightly lower than the control during the administration period although this did not achieve statistical significance. No effects were seen at the end of the recovery period.

CD rats: Group mean food consumption for treated animals was generally lower than controls during the administration period although this did not always attain statistical significance. Food consumption for the recovery animals returned to control levels by Week 8.

F344 rats: There was no evidence of any treatment related effects.

Organ weights

There was no evidence of any effects of glyphosate acid on the salivary gland weight at any time point in CD rats. On the contrary salivary gland weights were increased in the treated AP and F344 rats at the end of the administration period in comparison to control. While no effects were noted in the four or 13-week recovery AP animals, in F344 rats the salivary gland weights were still increased at these time points, although there was clear evidence of recovery.

Table B.6.8-13: Mean salivary gland weights at necropsy

Organ	AP		CD		F344	
	0	20000	0	20000	0	20000
	Terminal weight (g)					
Left salivary gland	0.652	0.740*	0.715	0.695	0.461	0.666**
Right salivary gland	0.523	0.659*	0.623	0.626	0.422	0.577*
	Weight after 4 week recovery					
Left salivary gland	0.748	0.703	0.844	0.742	0.488	0.555
Right salivary gland	0.639	0.623	0.701	0.637	0.428	0.505*
	Weight after 13 week recovery					
Left salivary gland	0.750	0.760	0.790	0.819	0.623	0.612
Right salivary gland	0.669	0.681	0.668	0.705	0.495	0.528

No macroscopic abnormalities were seen in salivary glands in any rat, either at the end of the administration period or after the four or 13-week recovery periods.

Treatment-related histological findings were confined to the parotid salivary gland and comprised alteration in the staining of the cytoplasm of the acinar cells. The affected cells appeared strongly basophilic and enlarged (recorded as basophilia of parotid acinar cells).

At the end of the four-week administration period this change was most prominent in F344 rats. All rats showed marked cytoplasmic basophilia that was diffuse, involving the whole of the parotid gland. However, no evidence of cell degeneration or necrosis was seen. Most of the control F344 rats also showed a minor degree of basophilia involving occasional acinar cells only.

The other two strains, AP and CD, both showed the same effect in the parotid gland after four weeks treatment but at a much reduced severity compared to the F344. In addition the distribution was different in that only small focal groups of acinar cells were affected in the AP and CD rats in contrast to the diffuse involvement seen in the F344. The effect was weakest in the CD rat.

The incidence data at the end of the administration period indicate that the background change varies in control rats in the three strains. None was seen in the AP controls, there was a single CD control rat with a minimal focal change, whereas 7 out of 8 F344 controls showed minor changes.

After four weeks recovery in the F344 strain the severity of the parotid basophilia was reduced to minimal or slight and affected small foci of acinar cells only. No changes were seen in the CD rats and only a single AP rat showed a minimal change. As an AP control rat showed changes at this time point this is considered not to be related to treatment.

After 13 weeks recovery no treatment related changes were seen in the AP and CD strains. Slightly more of the F344 rats showed minor focal changes compared to the corresponding control group but this may reflect variations in the background spontaneous change rather than a residual effect of treatment.

Table B.6.8-14: Histopathological findings in salivary glands

Finding	AP		CD		F344	
	0	20000	0	20000	0	20000
	Termination*					
Atrophy (marked)	0 / 8	0 / 8	1/8	0 / 8	0 / 8	0 / 8
Interstitial fibrosis (marked)	0 / 8	0 / 8	1/8	0 / 8	0 / 8	0 / 8
Basophilia of parotid acinar cells	0 / 8	8 / 8	1/8	7 / 8	7 / 8	8 / 8
	4 week recovery*					
Mononuclear cell infiltration (minimal)	0 / 8	1 / 8	0 / 8	0 / 8	1 / 8	0 / 8
Basophilia of parotid acinar cells	1 / 8	1 / 8	0 / 8	0 / 8	0 / 8	6 / 8
Mucous metaplasia of parotid (slight)	0 / 8	1 / 8	0 / 8	0 / 8	0 / 8	0 / 8
	13 week recovery*					
Mononuclear cell infiltration (minimal)	0 / 8	0 / 8	0 / 8	0 / 8	1 / 8	1 / 8
Atrophy (minimal)	0 / 8	0 / 8	0 / 8	0 / 8	1 / 8	0 / 8
Basophilia of parotid acinar cells	1 / 8	1 / 8	1 / 8	1 / 8	1 / 8	5 / 8

* number of animals affected / total number of animals examined

Conclusion by the Notifiers

Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slighter effects occurred in the AP and CD strains involving small foci of cells only.

Complete recovery of effects was apparent in AP and CD strains following the 4-week recovery period and significant recovery had occurred in the F344 strain. It is not clear whether the slightly higher incidence of minor focal changes in the salivary glands of the F344 strain after 13-week recovery was a residual effect of treatment or represented the random variation in the background incidence in this strain.

RMS comments:

The study is considered acceptable. Clear strain differences became obvious and might, to some extent, explain why salivary gland changes were observed in some but not all rat studies. However, it is still surprising that, e.g., histological lesions were seen in a long-term study in AP rats by Milburn (1996, TOX2000-1998) but not by Brammer (2001, ASB2012-11488) in the same laboratory although nearly identical dose levels had been employed. (However, the test material was of slightly lower purity in the Milburn study.)

It cannot be excluded that similar salivary gland effects as in rats would also occur in humans if exposure to glyphosate was high enough. F344 rats were rarely used for toxicological testing of glyphosate. Thus, it cannot be argued that a rat strain of particular sensitivity was employed in studies that were used for risk assessment. Furthermore, there is no proof that particularly sensitive F344 rats, with regard to salivary gland effects, were a less suitable model for man than other rat strains.

B.6.8.1.1 Further studies

Introduction by RMS:

In this section, different studies are compiled that cover various aspects of toxicology of glyphosate. Two studies were submitted as part of the GTF dossier, are summarised in Table B.6.8-15 and described in detail and commented by the RMS below. Amendments or corrections have been made where necessary.

In the 1998 DAR, some information on mechanisms of toxicity (██████████ 1992, TOX9552421; ██████████ 1987; TOX9552430) and a possible additive toxic effect of glyphosate with either dalapon or 2,4-D (██████████ 1987, TOX9551964) is given. The brief descriptions of these studies with conclusions obtained during previous evaluation are copied from the old DAR, subsequent to the new studies.

Table B.6.8-15: Overview on newly submitted mechanistic studies for effects other than on salivary glands (provided by the Notifiers)

Reference; Study identification; Owner	Type of study Species, Strain	Application Route (Dose)	Test substance	Purity [%]	Results
██████████ 1996; ASB2012-12054; Nufarm	Screening study for pharmacological effects, Sprague-Dawley Rat, ♂ + ♀ for <i>in vivo</i> investigations, isolated organs from rats and guinea pigs for <i>ex vivo</i> experiments	Gavage (5000 mg/kg bw, single dose), for <i>in vivo</i> experiments; guinea pig ileum exposed in an isolated organ bath; for examination of neuromuscular activity following injection into previously killed animals	Glyphosate technical	95.3	No haematological, electrocardiographic or behavioural/functional changes after oral administration; contractile response similar to that seen with known parasympathomimetic agents in isolated guinea pig ileum; no neuromuscular blocking activity on innervated rat gastrocnemius muscle
██████████ 2012; ASB2012-11521; Monsanto	Mice, B5C3F1 ♀	Diet 0, 500, 1500, 5000 ppm (0, 150, 449, 1448 mg/kg bw/day)	Glyphosate	95.11	No suppression of the humoral component of the immune system. No test-substance-related effects

Pharmacological activity (██████████ 1996)

Reference:

IIA, 5.10/03

Report:

██████████ (1996)

Glyphosate Technical: Pharmacology Screening Study in the Rat

Data owner: Nufarm

Study No.: 434/021

Date: 1996-06-28

Unpublished

ASB2012-12054

Guidelines:

JMAFF, 59 Nohsan No. 4200 (1985)

Deviations: not applicable
GLP: Yes
Acceptability: See RMS comment

Dates of experimental work: 1996-02-06 to 1996-04-04

Materials and methods

Test materials:

Identification: Glyphosate Technical
 Description: White powder
 Lot/Batch #: H95D161A
 Purity: 95.3 %
 Stability of test compound: No data given in the report.
 Vehicle: *in-vivo* 1% carboxymethylcellulose
ex-vivo (guinea pig) distilled water, krebs physiological buffer solution,
ex-vivo (guinea pig) physiological saline

Test animals:

in-vivo Species: Rats
 Strain: Sprague-Dawley (CD)
 Source: [REDACTED]
 Age: no data
 Sex: Males and females
 Weight at animal receipt (corrected by RMS): 176 - 200 g
 Acclimation period: At least 6 days
 Diet/Food: SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
 Water: Tap water, *ad libitum*
 Housing: By sex in groups of five in polypropylene cages with stainless steel grid floors.
 Environmental conditions: Temperature: 19 – 25 °C
 Humidity: 40 – 75 %
 Air changes: at least 15/hour
 12 hours light/dark cycle
ex-vivo Species: Guinea pig
 Strain: Dunkin Hartley
 Source: David Hall Ltd., Staffordshire, UK
 Age: no data
 Sex: Males
 Weight at animal receipt: 250 - 300 g

Acclimation period:	no data
Diet/Food:	Guinea Pig FDI Diet (Special Diets Services Ltd., Witham Essex, UK), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	By sex in groups of up to three in polypropylene cages with solid floors and sawdust bedding.
Environmental conditions:	Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: at least 15/hour 12 hours light/dark cycle
<i>ex vivo</i> (amended by RMS)	Species: Rats
Strain:	Sprague-Dawley (CD)
Source:	Charles River UK Ltd., Margate, Kent, UK
Age:	no data
Sex:	Males and females
Weight at animal receipt :	110 - 125 g
Acclimation period:	no data
Diet/Food:	SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	By sex in groups of five in polypropylene cages with stainless steel grid floors.
Environmental conditions:	Temperature: 19 – 25 °C Humidity: 30 – 70 % Air changes: at least 15/hour 12 hours light/dark cycle

Animal assignment and treatment of *in vivo* studies:

Three groups of five male and five female rats each received glyphosate technical at a dose level of 5000 mg/kg bw by oral gavage. The control group was similar sized receiving vehicle only. The dosing volume was 10 mL/kg bw. Approximately one hour after dosing control and treated animals were examined for either haematological changes, electrocardiographic changes or behavioural/functional changes.

Blood parameters

Blood samples were taken from all animals via a tail vein. The following parameters were evaluated: Haemoglobin (Hb), total erythrocyte count (RBC), haematocrit (Hct), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (WBC), platelet count (PLT) and clotting (Prothrombin) time (CT).

Cardiovascular system

After animals were anaesthetised, cardiac activity was assessed using an electrocardiograph. A limb lead was attached to each limb and connected to the electrocardiogram. The equipment was set to lead II measurement at a sensitivity of either 10 mm/mvolt or 5 mm/mvolt and a chart speed of 25 mm/second. The following parameters were evaluated: Heart rate, P-R interval, QRS interval, Q-T interval, P-amplitude, R-amplitude, T-amplitude.

Nervous system

Animals were placed individually in a purpose built arena and assessed for behaviour and response to various stimuli using a modified Irwin Screen. The following parameters were evaluated: Salivation, hypo/hyperthermia, skin colour, respiration, lacrymation, palpebral closure, pilo-erection, exophthalmia, gait, twitches, tremors, convulsions, abnormal behaviour, tail elevation, transfer arousal, urination, defaecation, vocalisation, finger approach, touch escape, tail & toe pinch, grasp response, auditory startle response, pupil response to light, palpebral reflex.

Animal assignment and treatment of *ex vivo* studies:

Guinea pig - Isolated ileum

Sections of ileum were dissected from previously untreated guinea pigs killed by cervical dislocation. and were transferred to a purpose built isolated organ bath containing Krebs buffer solution with a test substance concentration of 12 mg/mL (maximum solubility). The isolated ileum was connected to the lever arm of an isotonic transducer by a cotton ligature. The transducer was connected to a chart recorder. Contractions of the isolated ileum could then be recorded. Standard solutions of acetylcholine, a known agonist, were prepared and added to the volume of buffer solution used to bathe the isolated ileum. A maximum volume of 2 mL was used for all experiments to ensure the integrity of the tissue in the medium. The contraction response of isolated ileum was recorded for each concentration of acetylcholine to produce a standard curve. Between additions of each new concentration of acetylcholine, the buffer in the organ bath was flushed out and replaced by fresh buffer. The test material, dissolved in buffer, was added and its response compared with standards. Following initial results an antagonist (atropine) to the effects of acetylcholine was added together with the agonist. The results were then compared with the effects of an antagonist and the test material.

The following parameters were evaluated: Response to acetylcholine (agonist), test material, atropine (antagonist).

Rat - Gastrocnemius muscle

Previously untreated rats were killed by cervical dislocation. The abdomen was immediately dissected open and the dorsal aorta exposed. A butterfly needle was inserted into the dorsal aorta, near to the bifurcation in a posterior direction.

A volume of 0.3 mL of lithium heparin at a concentration of 10 mg/mL in sterile saline was injected into the dorsal aorta followed by 0.5 mL of sterile saline.

The experiment itself was performed on separate animals with doses of either the test material dissolved in sterile saline at a concentration of 12 mg/mL (maximum solubility) or of tubocurarine (positive control) that were injected into the dorsal.

The gastrocnemius muscle of the hind limb was exposed with the sciatic nerve intact. The gastrocnemius muscle was detached from the ankle joint and this area was ligated with cotton which was then attached to the lever arm of a transducer. The limb was held in place by a series of pins. An electrical stimulus of 12 volts was applied to the sciatic nerve and the muscle response was recorded. This action was repeated at approximately twelve second intervals until sufficient responses had been recorded.

The following parameters were evaluated: Response to injection of sterile saline, tubocurarine and test material.

Results and discussion

Blood parameters

There were no biologically significant differences, among the parameters measured, between treated and control animals.

Cardiovascular system

There were no biologically significant differences, among the parameters measured, between treated and control animals.

Nervous system

There were no biologically significant differences, among the parameters measured, between treated and control animals.

Guinea pig isolated ileum

The addition of acetylcholine to the medium containing the isolated guinea pig ileum resulted in contraction of the tissue in a concentration related response. Incubation with atropine sulphate immediately prior to addition of acetylcholine diminished or abolished the contraction response in a concentration related manner.

The addition of glyphosate technical at the maximum solubility in buffer also resulted in contraction of the ileum. The force of contraction was increased by an increasing volume of the test material in solution. Incubation with atropine sulphate prior to addition of glyphosate technical also resulted in the abolition of contractile response.

Rat- Gastrocnemius muscle

Injection of tubocurarine at a concentration of 25 mg/mL resulted in a significant diminution of the contractile response of the rat gastrocnemius muscle when the sciatic nerve was stimulated. There was no effect on muscle contraction when either glyphosate technical (12 mg/mL) or physiological saline was injected. The difference in force of response seen with glyphosate technical and physiological saline can be attributed to individual animal variation.

Conclusion by the Notifiers

At a maximum dose level of 5000 mg glyphosate technical/kg bw there were no effects seen from the *in vivo* screens performed. When administered to the isolated guinea pig ileum glyphosate technical caused a contractile response similar to that seen with known parasympathomimetic agents. Evaluation of innervated muscle response using showed that glyphosate technical, when administered at the maximum solubility concentration in physiological saline, did not cause any neuromuscular blocking activity.

RMS comments:

The study is considered acceptable although it will not contribute that much to risk assessment of glyphosate. The conclusion is agreed with. A parasympathomimetic activity of glyphosate, at least at a high dose level, was shown. This seems to be a bit contradictory to the adrenergic mechanism postulated by Chan and Mahler (1992, TOX9551954) for salivary gland effects but it must be taken into account that different tissues were investigated.

Immunotoxicity in mice (2012)**Reference:** IIA, 5.10/04**Report:** (2012)

Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice

Project No.: WI-10-460 (Study No.: WIL-50393)

Data owner: Monsanto

Date: 2012-03-21

not published

ASB2012-11521

Guidelines: US-EPA OPPTS 870.7800 (1998)**Deviations:** None**GLP:** Yes**Acceptability:** See RMS comment

Dates of experimental work: 2010-10-05 - 2010-11-17

Materials and methods

Test material:

Identification:

Glyphosate

Description:

White powder

Lot/Batch #:

GLP-0807-19475-T

Purity:

95.14% (dried)

Stability of test compound:

Expiry date: 2011-06-10

Vehicle and/
or positive control:

Basal diet

Cyclophosphamid monohydrate

Test animals:

Species:

Mouse

Strain:

B6C3F1/Crl

Source:

Age:

Approx. 37 days (on arrival)

Sex:

Female

Weight at dosing:

16.5 – 20.0 g

Acclimation period:

14 days

Diet/Food:

Certified Rodent LabDiet® # 5002 (meal) (PMI Nutrition International, LCC.), *ad libitum*

Water:

Tap water, *ad libitum*

Housing:

Individually in stainless steel, wire-mesh cages suspended above cage-board.

Environmental conditions: Temperature: 22 ± 3 °C
 Humidity: 50 ± 20 %
 Air changes: 10/hour
 12 hours light/dark cycle

Animal assignment and treatment:

In a 28-day oral immunotoxicity study groups of 10 female B6C3F1/Crl mice received daily dietary doses of 0, 500, 1500 and 5000 ppm glyphosate (equivalent to 0, 150, 449 and 1448 mg/kg bw/day).

A further group of 10 females were used as positive immunosuppressive control group. These mice received basal diet for 28 days and were treated with an intraperitoneal (IP) injection of 50 mg/kg bw/day once daily for four consecutive days (study days 24-27).

Test diets were prepared weekly and stored at room temperature. For the negative and positive control groups an appropriate amount of basal diet was weighed into a plastic storage bag. For the test substance groups 500 g of basal diet was weighed (pre-mixture). An appropriate amount of glyphosate was weighted into a mortar, mixed with a small amount of the pre-mixture basal diet, and ground until uniform. This admixture was transferred to a Hobart mixer and mixed with the remainder of the pre-mixture basal diet for five minutes. The resultant mixture was then transferred to a V-blender with a sufficient amount of basal diet to achieve the correct diet concentration and mixed for an additional 10 minutes using an intensifier bar during the first and last three minutes of mixing to ensure a homogeneous mixture. The test diets were prepared from the lowest to highest concentration. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 450 and 5500 ppm. Analyses for achieved concentrations on the test diets were done during study weeks 0 and 3.

Mortality

Each animal was checked for mortality or signs of morbidity twice a day during the treatment period, including weekends and public holidays.

Clinical observations

A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at once a week during the study period, beginning one week prior to randomisation, and on the day of scheduled necropsy.

Body weight

Individual body weights were recorded twice weekly, beginning approximately one week prior to randomization, at the time of animal selection for randomization, on study day 0, and just prior to the scheduled necropsy. Mean body weights and mean body weight changes were calculated for the corresponding intervals.

Food consumption and test substance intake

The quantity of food consumed was recorded for each animal weekly, beginning approximately one week prior to randomization, and just prior to the scheduled necropsy. Food intake was calculated as g/animal/day for the corresponding body weight intervals. The mean amounts of glyphosate consumed (mg/kg/day) per dose group were calculated from the mean food consumed (g/kg of body weight/day) and the appropriate target concentration of glyphosate in the food (mg/kg of diet).

Serum collection for possible IgM antibody analysis

For determination of the possible extent of the suppression of IgM antibody production blood samples were collected from all animals at scheduled necropsy and processed to serum. Following euthanasia by carbon dioxide inhalation, approximately 0.75 mL of blood was collected from the inferior vena cava of each mouse into a tube containing no anticoagulant and allowed to clot. Serum was obtained and aliquots of approximately 150 µL (including any remainder serum) were transferred to cryovials and stored frozen (approximately -70 °C).

Sacrifice and pathology

A complete necropsy was conducted on all animals at scheduled termination or on animals that died or were sacrificed during the study period. Any macroscopic findings were recorded. The following organ weights were determined from all animals surviving to scheduled termination: spleen and thymus.

Tissue samples were taken from the spleen and thymus. Spleen samples were placed in EBSS/HEPES buffer. Thymus samples were preserved in 10% neutral-buffered formalin.

Spleen processing for immunotoxicological evaluation

For the determination of the number of specific IgM antibody-forming cells directed towards sRBC an AFC assay, as a modification of the Jerne plaque assay (Jerne et al., 1963, 1974) was conducted.

Spleens were collected from all animals at the scheduled necropsy (study day 28) immediately following blood collection. Individual spleens were placed into individual tared tubes containing EBSS with 15 mM HEPES, supplemented with gentamicin as a bacteriostat, and maintained on ice. Each tube was then weighed to provide a “wet” weight for each spleen. Spleen samples from Groups 1-4 animals were randomized and coded for antibody-forming cell (AFC) analysis. Spleen samples from Group 5 were labelled as positive control samples for analysis. The spleen samples were placed on crushed ice until procession for AFC analysis.

The spleen samples were processed into single-cell suspensions. The cell suspensions were then centrifuged and resuspended in EBSS with HEPES. Spleen cell counts were performed using a Model Z1™ Coulter Counter®. Viability of splenocytes was determined using propidium iodide and the Coulter® EPICS® XL-MCL™ Flow Cytometer

Statistics

Body weight, body weight change, and food consumption data were subjected to a parametric one way ANOVA to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunnett's test was used to compare the test substance treated groups to the control group.

The positive control data were evaluated using the Student's t-Test and compared to the basal diet control group.

Organ weight (wet spleen and thymus), final body weight, and AFC data obtained were first tested for homogeneity of variances using the Bartlett's Chi Square test. Homogeneous data were evaluated using a parametric one-way ANOVA. When significant differences occurred, the treatment groups were compared to the basal diet control group using Dunnett's test. Non-homogeneous data were evaluated using a non-parametric ANOVA. When significant differences occurred, the treatment groups were compared to the basal diet control group using the Gehan-Wilcoxon test when appropriate. The Jonckheere's test was used to test for dose-related trends across the basal diet control and test substance treated groups. The

positive control data were evaluated using the Student's t Test and compared to the basal diet control group. The criteria for accepting the results of the positive control group included a statistically significant ($p \leq 0.05$) decrease in the response when compared to the response of the basal diet control group.

The AFC data were expressed as Specific Activity, IgM antibody forming cells per million spleen cells (AFC/10⁶ spleen cells), and as IgM Total Spleen Activity (AFC/spleen).

Results and discussion

Diet analysis

The achieved concentrations of glyphosate in the dietary preparation were in the range of 85.6 - 97.5% of nominal, and therefore within the acceptable range of 85 - 115 %. The diet formulations were homogeneous and stable for 10 days when stored at room temperature with the following exception. During homogeneity/concentration acceptability testing, the 450 ppm diet formulation was 83.1% of target. The 5500 ppm diet formulation was within acceptable range (90.8 %) but was considered low, therefore, calibration standards were prepared as matrix-based samples and a cross-validation was conducted. The diet formulations were reanalyzed using matrix-based calibration standards and met the testing facilities SOP acceptance criteria for homogeneity and concentration acceptability. Based on these results, the protocol-specified doses of test substance were offered to the animals. The test substance was not detected in the basal diet that was offered to the basal diet control (Group 1) and positive control (Group 5) groups.

Mortality

There were no mortalities observed during the study period.

Clinical observations

There were no test substance-related clinical findings.

Body weight

There were no test substance related

Food consumption

There were no test substance-related effects on food consumption noted.

Gross pathology

There were no test substance-related macroscopic effects.

Treatment with the positive control CPS produced a small thymus in three of the 10 animals. These changes were consistent with the known effects of CPS in female B6C3F1 mice.

Organ weights

There were no test substance-related effects on terminal body weights or on spleen or thymus weights (absolute or relative to final body weight) when the test substance-treated groups were compared to the basal diet control group.

Treatment with the positive control CPS produced statistically significantly lower spleen and thymus weights (absolute and relative to final body weight) when compared to the basal diet control group. These changes were consistent with the known effects of CPS in female B6C3F1 mice.

The results of final body and organ weight determinations are presented in the Table B.6.8-16 below.

Table B.6.8-16: Final body weight and organ weight data

Dose group	Body weight	Spleen		Thymus	
		weight	% body weight	weight	% body weight
	(g) [#]	(mg) [#]	(%) [#]	(mg) [#]	(%) [#]
1 (negative control)*	20.9 ± 0.3	85.3 ± 3.5	0.41 ± 0.02	44.3 ± 3.5	0.21 ± 0.02
2 (low)	20.6 ± 0.2	82.3 ± 4.6	0.40 ± 0.02	41.5 ± 1.9	0.20 ± 0.01
3 (mid)	21.6 ± 0.3	91.6 ± 6.5	0.42 ± 0.03	45.9 ± 2.7	0.21 ± 0.01
4 (high)	21.3 ± 0.2	86.0 ± 3.6	0.40 ± 0.02	42.0 ± 2.6	0.20 ± 0.01
5 (positive control)	21.5 ± 0.3	50.2 ± 3.2**	0.23 ± 0.02**	13.3 ± 0.8**	0.06 ± 0.01**

[#] Values presented the mean ± SD derived from the number of animals evaluated per dose group

** Statistically significant from negative control at $p \leq 0.01$

AFC assay

There were no test substance-related effects on spleen cell numbers, and in the functional evaluation of the IgM antibody-forming cell (AFC) response, treatment with glyphosate did not result in a statistically significant suppression of the humoral immune response when evaluated as either Specific Activity (AFC/10⁶ spleen cells) or Total Spleen Activity (AFC/spleen). There were no statistically significant differences nor any dose-related trends noted when the basal diet control and test substance-treated groups were compared.

Statistically significantly lower spleen cell numbers, mean specific activity, and mean total spleen activity values were noted in the positive control (CPS treated) group when compared to the basal diet control group. These effects were consistent with the known immunosuppressant effects of CPS and validated the appropriateness of the AFC assay.

The results of the AFC assay are summarised in Table B.6.8-17 below.

Table B.6.8-17: Results of AFC assay

Dose group	Spleen cells	IgM AFC / 10 ⁶ spleen cells	IgM AFC/spleen
	(x 10 ⁷) [#]	[#]	(x 10 ³) [#]
1 (negative control)*	11.29 ± 0.65	1160 ± 131	127 ± 11
2 (low)	11.45 ± 0.64	1273 ± 123	144 ± 16
3 (mid)	13.45 ± 0.24	1368 ± 163	190 ± 37
4 (high)	12.51 ± 0.66	1514 ± 204	195 ± 32
5 (positive control)	5.18 ± 0.53**	0 ± 0**	0 ± 0**

[#] Values presented the mean ± SD derived from the number of animals evaluated per dose group

** Statistically significant from negative control at $p \leq 0.01$

Conclusion by the Notifiers

Repeated dietary administration of glyphosate to females B6C3F1 mice did not suppress the humoral component of the immune system. The no-observed-effect level (NOEL) for suppression of the humoral immune response in female B6C3F1 mice offered glyphosate in the diet for 28 days was considered to be 5000 ppm (equivalent to 1448 mg/kg bw/day), the highest dietary concentration.

RMS comments:

The study is considered acceptable. For the parameters included, there was no evidence of immunotoxicity of glyphosate in mice up to a very high dose level.

Studies on mechanisms of toxicity and additive effects (copied from 1998 DAR, ASB2010-10302)

(1992, TOX9552421): Ammonium salt of glyphosate (MON-8750): General pharmacological study. Institute of Environmental Toxicology, Tokyo, Japan; Study no. IET 90-0149/ET-92-15). Dates of experimental work: 04 February 1992 - 02 March 1992. GLP: yes (self-certification of the laboratory). This report was submitted as part of the joint dossier of Monsanto and Cheminova.

The potential effects of MON 8750 (purity: 94.78%) on the nervous, respiratory and circulatory systems were assessed following single i.p. injection to male and female ICR mice at doses up to 5000 mg/kg bw and single i.v. injection to urethane-anesthetized and non-anesthetized male rabbits at doses up to 500 mg/kg bw. At the top dose levels, all mice died within 0.5 hours and all anesthetized rabbits within a few minutes after injection. Non-anesthetized rabbits survived i.v. application of 500 mg/kg bw although animals showed some neurological signs. In rabbits which died, heart rate was decreased and ECG changes have been noted. At the next lower dose levels (1250 mg/kg bw in mice or 125 mg/kg bw and 31.3 mg/kg bw in rabbits, respectively), transient symptoms like a decrease in blood pressure, reduced activity and neuromuscular signs were observed but cleared to normal values or behaviour within some hours at the latest. Respiratory rate was increased in surviving rabbits but decreased in anesthetized rabbits which died. It was concluded that an impact on cardiorespiratory functions is involved in acute toxicity. The lethal dose appears to be decreased under anesthesia.

(1987, TOX9551964): Synergism and potentiation in rats of glyphosate (tech.) of Excel Industries Ltd., Bombay. Study identification and dates of experimental work not given. GLP: no. This report was submitted independently by the notifiers Barclay and Luxan.

Glyphosate was administered to groups of 10 male Wistar rats as a single oral dose of 5000 mg/kg bw at a constant dose volume of 10 ml/kg in corn oil. Simultaneously, the animals received the compound 2,4-D sodium salt at dose levels of 376, 473, 596, 750, 944 or 1189 mg/kg bw. The second compound used for a potentiation experiment was dalapon at doses of 2500 and 5000 mg/kg bw. After simultaneous dosing, the rats were observed for 14 days for toxic symptoms and mortality. According to the study authors, no potentiation has been observed with glyphosate and dalapon. However, two out of ten rats died after application of 5000 mg/kg bw glyphosate and 5000 mg/kg bw dalapon. When both compound were administered alone, no mortalities occurred.

The simultaneous administration of glyphosate and 2,4-D sodium salt caused a markedly higher mortality in all dose groups. In the groups receiving glyphosate and doses of 596 mg 2,4-D sodium salt/kg bw and above, all animals died. When the latter compound was administered alone, 100% mortality has been reached only at 1189 mg/kg bw. Hence, at least an additive acutely toxic effect of glyphosate and 2,4-D can be assumed.

(1987, TOX9552430): Irritating effect of glyphosate, surfactant and Roundup on stomach and small intestine in dogs. Dep. of clinical medicine, University of Tsukuba, Japan. The study was submitted as part of the joint dossier of Monsanto and Cheminova.

The IPA salt of glyphosate, Roundup herbicide (41% IPA) and the surfactant MON 0818 (15% of which is contained in Roundup) and 0.25 N hydrochloric acid solution (control) were directly administered on the gastric and small intestinal mucosa of fasted male beagle dogs. The specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues. Direct application of Roundup®

herbicide, and the surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup formulation than with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused by 0.25 N hydrochloric acid.

Further Studies on mechanisms of toxicity and additive effects (submitted after 2000)

Song, H.-Y. et al. (2012, ASB2013-10531): *In vitro* cytotoxic effect of glyphosate mixture containing surfactants.

The authors investigated whether glyphosate influences the cellular toxicity of the surfactants polyoxyethylene tallow amine (TN-20) and polyoxyethylene lauryl amine ether (LN-10) on the mouse fibroblast like cells, alveolar epithelial cells and a heart cell line. The cytotoxicity of TN-20 and LN-10 (0.4-100 µM), in the presence or absence of glyphosate was determined by assessing membrane integrity. TN-20 toxicity was significantly lower in the presence of 50 µM glyphosate for the fibroblast-like cell, for the alveolar cells and for the heart cell line compared to that of TN-20 alone. The cellular toxicity of LN-10 towards the fibroblast-like cells was found to be increased in the presence of 50 µM glyphosate. The authors conclude that these results suggest that the mixture toxicity may be a factor in glyphosate-surfactant toxicity in patients with acute glyphosate herbicide intoxication.

B.6.8.3 Studies in farm animals

Table B.6.8-18: Summary of studies in farm animals

Reference; Study identification; Owner	Type of study Species, Sex	Application route (Dose)	Test material	Purity [%]	Results
1987; TOX9552422; Monsanto	Acute oral toxicity, Goat ♀	Gavage (0, 1980, 3090, 4620, and 10 000 mg/kg bw)	Glyphosate	98.7	LD ₅₀ = 3530 mg/kg bw
1987; TOX9552423; Monsanto	Acute oral toxicity, Goat ♀	Gavage (0, 1400, 4290, 5360, 6700, and 10 000 mg/kg bw)	Glyphosate isopropyl-amine salt	62.5 (46.2% glyphosate)	LD ₅₀ = 5700 mg/kg bw [CI 95%, 3.73–8.71]
1987; TOX9552424; Monsanto	Subacute oral toxicity (7 d), Cow ♀	Gavage (540, 830, 1290, and 2000 mg/kg bw/d; 1000 mg/kg bw/d in pre-test)	Glyphosate isopropyl-amine salt	62.4 (46.2% glyphosate)	MLD = 1290 mg/kg bw/d; LOAEL = 830 mg/kg bw/d; NOAEL = 540 mg/kg bw/d
1987; ASB2010-8131; Monsanto	Subacute oral toxicity (7 d), Cow ♀	Gavage (400, 500, 630, and 790 mg/kg bw/d; 1000 mg/kg bw/d in pre-test)	Glyphosate isopropyl-amine as Roundup® formulation MON2139	41.1 (30.5% glyphosate)	MLD = 790 mg/kg bw/d; LOAEL = 500 mg/kg bw/d; NOAEL = 400 mg/kg bw/d

MLD: minimum lethal dose

B.6.8.3.1 Acute toxicity in goats

Study with glyphosate acid

Reference: IIA, 5.10/05

Report: [REDACTED] (1987) The acute toxicology of glyphosate in female goats, [REDACTED]

Data owner: Monsanto

Report No.: VT-80-450

Date: 1987-03-23

TOX9552422

Guidelines: Non-guideline study

Deviations: Not applicable.

GLP: No

Acceptability: See RMS comments

Remark: The goats used in this study were *a priori* not tested for pregnancies (in fact, at least three of the goats employed for the study were pregnant) and, thus, females of different hormonal and physiological status were used. Further, weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous.

Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

Dates of experimental work: 1980-12-22 to 1981-05-05

Material and methods

Test material: Glyphosate

Description: white crystalline solid

Lot/Batch #: XHJ-64, NBP1494248

Purity: 98.7 % (N-phosphonomethyl glycine)

Stability of test compound: Stable for > 1 year (protocol date: 1980-08-28)

Vehicle and/or positive control: Water

Test animals: Species: Goat, Strain: not applicable (Spanish goats)

Source: [REDACTED]

Age: 8 months–4 years

Sex: female

Weight at dosing: 26.4 ± 7.2 kg

Acclimation period: 30 days

Diet/Food: During the initial acclimatization (outdoor): bermudagrass hay and commercial goat meal containing not less than 16 % crude protein [Purina ® Goat Chow ® (Coarse) (Ralston Purina Company, Gonzales, TX, USA)]. After the initial acclimatisation (indoor) the goats were fed bermudagrass only.

Water: not specified

Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study

Environmental conditions: not stated

Study design and methods

Dose 1980, 3090, 4620, and 10 000 mg/kg bw

4 control groups of 5 goats each

Application route: Oral, gavage

Application volume: 500 mL/goat

Fasting time: not stated

Group size: 5

Post-treatment observation period: 14 days

Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology

In life dates: not stated

Animal assignment and treatment:

Groups of female Spanish goats received the test item, glyphosate, at dose levels of 10 000, 4620, 3090, and 1980 mg/kg bw by oral gavage in a sequential manner. Initially, the test item was given to a group receiving 10 000 mg/kg/bw and subsequent dosages were selected based on the observed responses. Control animals were treated with tap water. All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Results and discussion

Mortality

All animals treated with 10 000 and 4620 mg/kg bw glyphosate died. 1/5 animals treated with 3090 mg/kg bw also died.

The results are summarised in the following table.

Table B.6.8-19: Mortality, survival time and animals used

Dose (mg/kg bw)	Toxicological result			Onset of death after	Mortality (%)
	Dead animals	Animals with toxic signs	N		
10 000	5	5	5	3.9–19.2 h	100
4620	5	5	5	2.4–32.2 h	100
3090	1	5	5	71.7 h	20
1980	0	4	5		0
0	0	0	20		0

N = Total number of animals in group

The median lethal dose (LD₅₀) was calculated to be
LD₅₀, goat, oral = 3530 mg/kg bw.

Clinical observations

Clinical signs of toxicity included colic, diarrhea, depression, and ataxia. Most of these signs were observed at all dosage levels except the 1980 mg/kg bw level at which only diarrhea was observed.

In detail: Clinical signs of goats that died included cessation of feeding activity, abdominal distress, depression, ataxia, mild diarrhea, and, shortly prior to death, recumbency. Toxic tubular nephrosis was the only consistent histopathological lesion observed in goats that died. Clinical signs of surviving goats included decreased feed consumption, diarrhea, and body weight loss.

Clinical signs of goats treated with the minimum dose included decreased feed consumption and diarrhea.

All clinical signs were absent at the end of the experiment (14–15 d).

One control goat gave birth to a single live kid approximately one hour before the time of treatment. Another control goat gave birth to one normal and one small and very weak kid on day 4 of the experiment.

One goat of the 4.62 g/kg bw-treated animals gave birth to two full-term kids during the night before dosing.

Table B.6.8-20: Prominent clinical observations in female Spanish goats given glyphosate

Dose (mg/kg bw)	N	Observations (Number of goats affected)
10 000	5	CFA (5), apparent colic (2), depressed demeanor (2), slight ataxia (3), recumbency (4), death (5)
4620	5	CFA (5), apparent colic (2), depressed demeanor (2), ataxia (2), labored breathing (2), recumbency (4), diarrhea (1), death (5)
3090	5	<u>fatally poisoned goat (1):</u> CFA, diarrhea, apparent colic, subdued demeanor, thirst, ataxia, recumbency, nystagmus, death <u>survivors (4):</u> decreased food consumption (4), apparent colic (2), diarrhea (4)
1980	5	Decreased food consumption (4), diarrhea (3)

CFA: cessation of feeding activity

Body weight

Most animals, which died while on study, exhibited losses in weight immediately prior to death.

Of the animals that survived until terminal sacrifice, those in the 3090 mg/kg bw group had mean body weights less than their respective control group. Goats in the 1980 mg/kg bw group had mean body weights similar to their respective control group.

Food consumption was not precisely measured, but cessation of feeding activity was observed in all treatment groups.

Table B.6.8-21: Survival time, body weights and carcass weights (control groups not pooled)

Dose (mg/kg bw)	Mean survival time (h)	Mean body weight (kg)				Mean carcass weight		
		N	Initial	Day 7	Day 14	N	Weight (kg)	Day
10 000	13.7	5	24.68	–	–	2	26.82	0
Control	S	5	24.36	23.22	23.68	–	–	–
4620	16.46	5	29.46	–	–	5	28.78	0–1
Control	S	5	27.76	26.81	26.63	–	–	–
3090	71.7*	5**	25.81	21.04	22.79	1	30.62	3
Control	S	5	28.58	29.89	28.12	–	–	–
1980	S	5	24.72	22.77	23.54	–	–	–
Control	S	5	25.95	24.95	25.08	–	–	–

–: not applicable

S: all animals survived

* One animal died at day 3 the others survived

** N = 4 for day 7 and 14.

Necropsy

Pulmonary edema to some degree was noted in several animals and was judged to be a terminal event.

The results of the post-mortem observations are summarised in the following table.

Table B.6.8-22: Summary of major gross pathologic diagnoses (animals affected/total)

Dose (mg/kg bw)	Died/sacrificed	Body as a whole	Respiratory	Cardiovascular	Gastro-intestinal	Urogenital
10 000	5/0	NGPD	Pulmonary edema (4/5) Pneumonia (1/5)	NGPD	NGPD	NGPD
4620	5/0	Serous atrophy of fat (1/5)	Pulmonary edema (4/5)	NGPD	Hepatic atrophy (1/5)	Macerated fetus (1/5)
3090	1/4	Serous atrophy of fat (1/5)	Pneumonia (1/5)	Pericarditis (1/5)	Fatty liver (1/5) Rumen haemorrhage (1/5)	Pallor, kidneys (4/5) Renal hypertrophy (1/5) Endometritis (2/5) Fetal death (1/5)
1980	0/5	NGPD	Pulmonary edema (3/5) Pneumonia (1/5)	NGPD	Fatty liver (1/5) Chronic hepatitis (1/5)	Renal atrophy (1/5)
Controls	0/4*	Minimal fat stores (1/4)	Pulmonary edema (2/4)	NGPD	NGPD	Pallor kidney (2/4) Cystitis (1/4) Metritis (1/4) Endometritis (1/4)

NGPD: No gross pathologic diagnosis

* only one randomly selected goat from each of the 4 control groups subjected to *post mortem* examination

Histopathology

Histological examination was performed only on heart, liver, kidney, spleen and other tissues with grossly visible lesions from animals given glyphosate at 4620 and 3090 mg/kg bw and of 4 sacrificed control animals.

The most consistent finding in fatally poisoned animals was mild to severe tubular nephrosis. 3090 mg/kg bw-treated animals that survived lack such lesions.

4/5 fatally poisoned goats had mild fatty change in the liver.

Clinical biochemistry and haematology

Elevation of blood urea nitrogen concentration, serum creatinine concentration, and numbers of circulating segmental neutrophils were the most consistent laboratory findings in goats given glyphosate. These changes were observed at all dosage levels used in this study.

Clinical laboratory findings were almost universally within or near control limits for surviving glyphosate-treated goats at the end of the experiment (day 14).

The results are summarized in the following tables.

Table B.6.8-23: Biochemical and haematological measurements of goats that died (min–max values)

Measurement (units)	Reference value	Control ¹	Death in less than 4 h (1 goat) ²	Death in 15–20 h (3 goats) ³	Death in 32–72 h (2 goats) ⁴	Death in 72 h (1 goat) ⁵
BUN (mg/dL)	10.0–20.0	2.3–21.3	12.7	16.2–25.0 ⁺⁺	16.7–35.2 ⁺⁺	55.5 ⁺⁺
SC (mg/dL)	1.0–1.8	0.7–1.7	1.5	2.7–3.3 ⁺⁺	4.0–6.0 ⁺⁺	14.1 ⁺⁺
GLU (mg/dL)	50.0–75.0	43–80	407 ⁺⁺	48–57	35–38	268 ⁺⁺
Na (mg/dL)	327–356	311–354	303 ⁺⁺	333–335	313–321	327
K (mg/dL)	13.6–26.1	12.7–21.2	44.8 ⁺⁺	21.8–31.5	15.2–20.5	12.9
Ca (mg/dL)	8.9–11.7	8.0–15.7	18.9 ⁺⁺	15.2–18.6 ⁺⁺	8.7–10.3	6.3 ⁺⁺
P (mg/dL)	2.9–7.3	3.5–11.3	16.0 ⁺⁺	5.4–9.5	8.8–17.0 ⁺⁺	21.6 ⁺⁺
Mg (mg/dL)	2.8–3.6	1.30–3.20	4.01 ⁺⁺	2.87–3.61 ⁺⁺	1.98–3.07	2.38
SGOT (IU/L)	167–513	31–151	78	49–126	60–85	129
LDH (IU/L)	123–392	108–385	295	172–543 ⁺⁺	268–288	694 ⁺⁺
SACH (IU/L)	---	319–478	426	272–475	266–287	324
ALKP (IU/L)	93–387	21–902	124	108–430	59–255	350
CPK (IU/L)	20–42	44–878	213	124–184	59–173	3285 ⁺⁺
GGT (IU/L)	---	12–42	---	23–26	20–21	39
WBC ⁵	4.0–15.0	5.5–24.8	14.5	22.5–28.0 ⁺⁺	13.5–27.0 ⁺⁺	57.3 ⁺⁺
SEGS ⁵	1.6–7.5	1.4–10.4	7.3	10.8–18.2 ⁺⁺	4.9–14.9 ⁺⁺	38.4 ⁺⁺
BANDS ⁵	---	0–1.4	2.8 ⁺⁺	6.4–7.6 ⁺⁺	4.1–10.0 ⁺⁺	11.5 ⁺⁺
LYMPHS ⁵	1.8–9.0	2.4–15.4	3.8	3.1–7.0	1.4–4.5	5.7
EOS ⁵	0.1–1.5	0–1.2	0.1	0	0	0
MONO ⁵	0.1–0.9	0–0.9	0.6	0.2–0.5	0.1–0.8	1.7
BASO ⁵	0–0.2	0–0.5	0	0–0.3	0	0
PCV(%)	19–40	23–42	26.0	31.0–40.0	27.0–28.0	39.0
HGB (g/dL)	8–16	8.1–16.2	8.6	9.4–12.3	8.2–11.0	15.4
RBC ⁶	7–21	13.3–29.5	13.6	19.0–24.0	14.0–15.9	21.4
MCV (fL)	15–39	13.0–21.0	19.0	14.0–17.0	16.0–18.0	18.0
MCH (pg)	5.3–8.4	4.4–7.8	6.3	4.7–5.1	5.9–6.9	7.2
MCHC (g/dL)	32–40	26.9–40.8	33.1	30.3–30.8	30.4–39.3	39.5
TSP (g/dL)	6.4–7.0	5.0–8.2	8.9 ⁺⁺	5.7–6.3	5.6–6.1	6.4

¹: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)

²: Goat sampled at death 3.8 h after receiving 10.0 g/kg

³: Goats sampled 11 h after receiving 10.0 g/kg

⁴: Goats sampled 24 h after receiving 4.62 or 3.09 g/kg

⁵: Goat sampled at death 72 h after receiving 3.09 g/kg

⁺⁺: One or more values outside of control limits

Table B.6.8-24: Biochemical and haematological measurements of surviving goats (min–max values)

Measurement (units)	Reference value	Control ¹	Sign of intoxication observed (4 goats) ²		No signs of intoxication observed (5 goats) ³	
			days 1 and 3	days 7 and 14	days 1 and 3	days 7 and 14
BUN (mg/dL)	10.0–20.0	2.3–21.3	15.1–45.7 ⁺⁺	3.1–26.4 ⁺⁺	5.2–54.5 ⁺⁺	7.0–31.3
SC (mg/dL)	1.0–1.8	0.7–1.7	1.8–5.3 ⁺⁺	0.8–1.6	0.8–5.0 ⁺⁺	0.8–1.3
GLU (mg/dL)	50.0–75.0	43–80	36–87	51–87	55–100	59–98
Na (mg/dL)	327–356	311–354	317–331	301–332	317–347 ⁺⁺	319–350 ⁺⁺
K (mg/dL)	13.6–26.1	12.7–21.2	6.7–18.1	9.4–17.0	12.3–18.3	11.9–18.2
Ca (mg/dL)	8.9–11.7	8.0–15.7	6.2–10.3 ⁺⁺	7.9–10.2	6.1–10.1	8.5–11.4
P (mg/dL)	2.9–7.3	3.5–11.3	4.4–22.4 ⁺⁺	4.1–8.0	3.7–9.0	5.3–8.7
Mg (mg/dL)	2.8–3.6	1.30–3.20	1.53–2.48	1.53–2.38	1.98–3.22	1.29–2.38
SGOT (IU/L)	167–513	31–151	44–69	47–229 ⁺⁺	40–91	46–106
LDH (IU/L)	123–392	108–385	100–233	127–415 ⁺⁺	145–276	144–226
SACH (IU/L)	---	319–478	231–447	221–390	293–440	291–445
ALKP (IU/L)	93–387	21–902	23–273	27–205	41–143	40–211
CPK (IU/L)	20–42	44–878	83–187	73–245	84–264	81–158
GGT (IU/L)	---	12–42	23–34	22–36	21–31	19–34
WBC ⁵	4.0–15.0	5.5–24.8	17.5–41.5 ⁺⁺	8.0–19.5	11.0–22.4	8.0–17.5
SEGS ⁵	1.6–7.5	1.4–10.4	11.5–30.7 ⁺⁺	2.4–14.8 ⁺⁺	4.1–17.5 ⁺⁺	2.0–7.2
BANDS ⁵	---	0–1.4	0.2–7.5 ⁺⁺	0.1–0.8	0–0.7	0–1.4
LYMPHS ⁵	1.8–9.0	2.4–15.4	2.4–5.4	2.9–12.2	4.0–6.9	5.0–10.2
EOS ⁵	0.1–1.5	0–1.2	0–0.2	0–0.3	0–0.3	0–0.7
MONO ⁵	0.1–0.9	0–0.9	0–1.0	0.1–0.5	0.1–0.2	0.1–0.4
BASO ⁵	0–0.2	0–0.5	0–0.2	0–0.3	0–0.3	0–0.3
PCV(%)	19–40	23–42	30.0–37.0	24.0–38.0	30.0–39.0	28.0–39.0
HGB (g/dL)	8–16	8.1–16.2	11.0–13.0	8.9–12.8	8.3–12.6	9.3–12.2
RBC ⁶	7–21	13.3–29.5	17.4–22.4	13.3–20.3	15.0–24.5	16.6–23.6
MCV (fL)	15–39	13.0–21.0	14.0–18.0	15.0–20.0	13.0–19.0	14.0–19.0
MCH (pg)	5.3–8.4	4.4–7.8	5.6–6.9	5.6–7.1	4.8–6.6	4.4–6.8
MCHC (g/dL)	32–40	26.9–40.8	34.2–40.0	29.1–39.3	31.1–35.3	26.9–34.9
TSP (g/dL)	6.4–7.0	5.0–8.2	6.5–7.5	5.5–7.5	6.0–7.3	6.0–7.4

¹: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)

²: Goats given 3.9 g/kg

³: Goats given 1.98 g/kg

⁺⁺: One or more values outside of control limits

Conclusion (Notifier)

The median lethal dose (LD₅₀) was calculated to be LD₅₀, goat, oral = 3530 mg/kg bw.

RMS comments: The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is good and it is suitable to provide additional information about acute oral toxicity in a ruminant species. The extent of investigations exceeded that one in acute toxicity studies in laboratory rodents. However, pathology was confined to four of a total of 20 control animals. In addition, assessment was made a bit difficult by the fact that sometimes data obtained in the four control groups were pooled and sometimes reported separately. The conclusion of the notifier is agreed with. Acute oral toxicity of glyphosate in goats was low although the goat appeared more sensitive than the rat, both with regard to dose levels causing mortality and severity of effects.

Study with glyphosate isopropylamine salt

Reference: IIA, 5.10/06

Report: [REDACTED] (1987) The acute toxicology of the isopropylamine salt of glyphosate in female goats

Data owner: Monsanto

Report No.: VT-80-451

Date: 1987-04-16

TOX9552423

Guidelines: Non-guideline study

Deviations: Not applicable

GLP: Yes (*Not checked by RMS. Not compulsory when the study was performed.*)

Acceptability: See RMS comments below.

Remark: The goats used in this study were *a priori* not tested for pregnancies (at least two goats, one each in the low dose and the control groups, had been pregnant because they aborted) and, thus, females of different hormonal and physiological status were used.

Further, weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous.

Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

Dates of experimental work: 1980-12-22 to 1981-05-08

Material and methods

Test material: MON 0139 (isopropylamine salt of glyphosate/
N-phosphonomethyl glycine isopropylamine)

Description: amber liquid

Lot/Batch #: LURT 08020

Purity: 62.5 % (N-phosphonomethyl glycine isopropylamine)

46.2 % glyphosate/N-phosphonomethyl glycine

Stability of test compound: Stable for > 1 year (protocol date: 1980-08-28)

Vehicle and/or positive control: water

Test animals:

Species: Goat

Strain: not applicable

(Spanish goats, the term is used to distinguish range meat goats from Angoras and dairy breeds. Most of the same origin as the Mexican Criollo but they may show traces of Nubian and Toggenburg blood.)

Source: [REDACTED]

Age: 8 months–4 years

Sex: Female

Weight at dosing: 27.1 ± 7.0 kg

Acclimation period: 30 days

Diet/Food: During the initial acclimatisation (outdoor): bermudagrass hay and commercial goat meal containing not less than 16 % crude protein (Purina ® Goat Chow ® (Coarse))

(Ralston Purina Company, Gonzales, TX, USA). After the initial acclimatization (indoor) the goats were fed bermudagrass only.

Water: Fresh water

Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study

Environmental conditions: not stated

Study design and methods

Animal assignment and treatment

Dose 1400, 4290, 5360, 6700, and 10 000 and mg/kg bw

Application route: Oral, gavage

Application volume: 500 mL/goat

Fasting time: not stated

Group size: 5

Four control groups of five animals each included

Post-treatment observation period: 14 days

Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology

In life dates: not stated

Animal assignment and treatment:

Groups of females Spanish goats received the test item, MON 0139, at dose levels of 10 000, 6700, 5360, 4290, and 1400 mg/kg bw by oral gavage in a sequential manner. Initially, the test item was given at 10 000 mg/kg/bw and subsequent dosages were selected based on the observed responses. Control animals were treated with tap water. All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Results and discussion

Mortality

Treatment with 10 000, 6700, 5360, 4290, and 14000 mg/kg bw MON 0139 resulted in 5/5, 3/5, 2/5, 2/5, and 0/5 deaths, respectively. In the four control groups, there were also no deaths.

The results are summarised in the following table.

Table B.6.8-25: Mortality, survival time and animals used.

Dose (mg/kg bw)	Toxicological result			Onset of death after	Mortality (%)
	Dead animals	Animals with toxic signs	N		
10 000	5	5	5	15.6–42.0 h	100
0	0	0	5		0
6700	3	5	5	37.0–64.0 h	60
0	0	0	5		0
5360	2	5	5	47.5–49.0 h	40
0	0	0	5		0
4290	2	5	5	53.2–85.1 h	40
0	0	0	5		0
1400	0	2	5	--	0
0	0	0	5		0

N: Number of animals

The median lethal dose (LD₅₀) was calculated to be LD₅₀, goat, oral = 5700 mg/kg bw [CI 95 %, 3.73–8.71].

Clinical observations

Clinical signs of toxicity included colic, diarrhea, taxia and weakness. These signs of toxicity were observed at most dosage levels above 1400 mg/kg bw. Additional symptoms suggestive of central nervous system involvement were observed at dosages of 4290 mg/kg bw and above, including tremors, convulsions, and unusual behaviour.

In detail:

Clinical signs of goats that died included decreased food consumption, abdominal distress, ataxia and, shortly prior to death, recumbency.

One goat that died and one surviving goat each displayed an unusual “collapsing syndrome” of apparent neurological origin approximately 2 days after receiving MON 0139 while other goats displayed various other neurological signs.

One surviving goat developed extensive ulceration of the tongue and oral mucosa. These lesions healed completely by the end of the 14-day observation period.

Table B.6.8-26: Prominent clinical observations in female Spanish goats given glyphosate MON 0139

Dose (mg/kg bw)	N	Observations (Number of goats affected)
10 000	5	<u>non-survivors (5)</u> : DFC (5); apparent colic (5); ataxia, shaking and jerking movements (2); depression (2); head bobbing (2); nystagmus (1); recumbency (4); diarrhea (1); paddling (2); mild convulsions (2)
6700	5	<u>non-survivors (3)</u> : DFC (3); apparent colic (2); diarrhea (1); bloating (3); ataxia (3); “saw-horse” stance (2); tremor (1); recumbency (3); nystagmus (2); jerky movements (2); blinking (1); terminal clonic-tonic activity (3) <u>survivors (2)</u> : DFC (2); bloating (1); abdominal distention (1); tremor (2); “collapsing syndrome” (1); diarrhea (2). (Abortion seen in one control goat)
5360	5	<u>non-survivors (2)</u> : DFC (2); diarrhea (1); ataxia (2); “saw-horse” stance (1); salivation (1); recumbency (1); tremor (1); “star-gazing” trance (1) <u>survivors (3)</u> : DFC (3); diarrhea (3)
4290	5	<u>non-survivors (2)</u> : DFC (2); ataxia (1); “collapsing syndrome” (1); “chewing convulsions” (1); recumbency (2); salivation (1); terminal opisthotonus-like convulsions (1) <u>survivors (3)</u> : DFC (3); diarrhea (3); apparent colic, salivation, lethargy, ulceration of oral mucosa (1)
1400	5	Minimal or no ill effects seen; reduced urination; abdominal distention (2) (Abortion seen in one control animal)

DFC: Decreased food consumption

Body weight

No statistically significant differences in body weight gain were observed between the groups treated with MON 0139 and their respective control groups.

Food consumption, although not precisely measured, was greatly reduced following treatment with MON 0139 at dosages of 4290 mg/kg bw and above. For most groups, feeding activity gradually increased, reaching normal levels by the end of the study.

Necropsy

No lesions considered to be treatment-related were noted at gross necropsy.

Table B.6.8-27: Summary of major gross pathologic diagnoses (animals affected/total)

Dose (mg/kg bw)	Died/sacrificed	Body as a whole	Respiratory	Cardiovascular	Gastro-intestinal	Urogenital
10 000	5/0	NGPD	pulmonary edema (2/5)	NGPD	NGPD	NGPD
6700	3/2	NGPD	pulmonary edema (1/5)	NGPD	bloat (2/5)	pale kidneys (1/5) renal hypertrophy (1/5)
5360	2/3	minimal fat stores (1/5)	pulmonary edema (3/5)	NGPD	fatty liver (4/5)	pale kidneys (2/5)
4290	2/3	NGPD	pulmonary edema (3/5)	NGPD	fatty liver (1/5) gallbladder edema (1/5)	pale kidneys (2/5) renal hypertrophy (1/5)
1400	0/5	minimal fat stores (1/5)	pneumonia (1/5) pulmonary edema (1/5)	pericarditis (1/5)	fatty liver (1/5)	metritis (1/5) pregnancy (1/5)
Control	0/4*	minimal fat stores (1/4)	pulmonary edema (2/4)	NGPD	NGPD	pale kidneys (2/4) mild cystitis (1/4) metritis (1/4) endometritis (1/4)

NGPD: No gross pathologic diagnosis

*: 1 goats of each of the four control groups was subjected to *post mortem* investigation

Histopathology

Mild to severe tubular nephrosis was the only consistent histopathological lesion observed in goats that died. However, this tubular nephrosis was not observed in goats that survived until the end of the experiment.

Tubular nephrosis is considered to be diagnostically significant for goats that died a few days after an appropriate level of exposure.

These lesions may have contributed to the observed elevations in blood urea nitrogen (BUN) and serum creatine (SC).

Ischemic hippocampal neurons were observed in the brain of one goat that had displayed the so-called "collapsing syndrome".

Clinical biochemistry and hematology

Slight to moderate elevations in blood urea nitrogen (BUN) and serum creatine concentrations (SC) were observed in all animals that died during the study. These findings may be related to the histopathologic kidney lesions observed in these animals.

Slight elevations in serum glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) activity were also observed in terminal animals immediately prior to death.

No other diagnostically or toxicologically significant changes were observed.

None of the biochemical parameters measured appeared to be involved in, or indicate the cause of, the unusual neurological manifestations seen in some goats receiving MON 0139.

The results are summarized in the following tables.

Table B.6.8-28: Biochemical and haematological measurements of goats that died (min–max values)

Measurement (units)	Reference value	Control ¹	Surviving < 49 h		Surviving > 50, < 90 h	
			Sampled 12–14 h before death (2 goats) ²	Sampled < 4 h before death (5 goats) ³	Sampled at 24 h post-treatment (4 goats) ⁴	Sampled 6–16 h before death (3 goats) ⁵
BUN (mg/dL)	10.0–20.0	2.3–21.3	26.7–44.1	31.5–62.4	13.8–22.0	44.2–111.4
SC (mg/dL)	1.0–1.8	0.6–1.7	4.5–6.1	3.3–5.8	1.4–2.9	5.1–6.7
GLU (mg/dL)	50.0–75.0	37–80	120–164	17–65	74–173	57–196
Na (mg/dL)	327–356	311–349	347–376	327–396	331–343	327–345
K (mg/dL)	13.6–26.1	13.4–21.2	16.2–16.5	10.9–23.2	10.3–16.9	9.0–11.0
Ca (mg/dL)	8.9–11.7	8.0–13.4	8.7–11.5	8.5–9.8	8.6–11.3	7.2–8.6
P (mg/dL)	2.9–7.3	4.1–11.9	2.9–16.0	3.5–10.0	3.9–6.4	2.9–5.9
Mg (mg/dL)	2.8–3.6	1.3–3.4	3.0–3.9	2.3–4.1	2.1–3.5	3.0–4.0
SGOT (U/L)	167–513	31–151	99–100	128–459	58–107	105–145
LDH (U/L)	123–392	145–385	303–310	394–880	220–298	266–394
SACH (U/L)	---	240–496	405–461	388–536	344–459	409–471
ALKP (U/L)	93–387	31–902	69–283	76–282	97–422	173–476
CPK (U/L)	20–42	44–878	144–185	181–2631	110–149	183–1145
GGT (U/L)	---	12–60	19–26	23–38	30–35	30–38
WBC ⁵	4.0–15.0	5.5–17.5	12.0–14.0	3.7–21.5	9.0–23.0	10.0–18.0
SEGS ⁵	1.6–7.5	1.4–10.4	3.1–7.0	1.3–4.8	4.1–16.6	1.8–6.9
BANDS ⁵	---	0–1.4	3.6–5.9	1.3–12.1	0–4.8	1.4–9.7
LYMPHS ⁵	1.8–9.0	2.4–9.2	2.6–2.8	1.0–6.5	1.4–4.9	1.7–4.4
EOS ⁵	0.1–1.5	0–1.4	0–0	0–0	0–0.1	0–0
MONO ⁵	0.1–0.9	0–0.9	0.4–0.6	0–0.9	0.1–0.4	0.1–0.5
BASO ⁵	0–0.2	0–0.8	0–0	0–0	0–0.1	0–0
PCV(%)	19–40	23–47	26–49	33–45	34–45	31–42
HGB (g/dL)	8–16	7.8–16.7	9.6–15.6	10.6–15.6	10.8–14.4	10.6–14.8
RBC ⁶	7–21	12.8–24.2	15.6–23.8	16.0–26.9	19.0–24.5	16.7–22.9
MCV (fL)	15–39	15–21	17.0–18.0	15.0–20.0	15.0–19.0	15.0–18.0
MCH (pg)	5.3–8.4	4.6–7.8	6.2–6.6	4.8–7.3	5.5–6.5	5.4–6.5
MCHC (g/dL)	32–40	28–41	31.8–36.9	31–35	32–37	32–35
TSP (g/dL)	6.4–7.0	5.0–8.2	6.7–8.6	5.8–7.3	7.2–7.7	6.2–7.7

¹: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)

²: Goats treated with 10 000 and 5360 mg/kg bw

³: Goats treated with 10 000 (n = 3), 6700 (n = 1), and 5360 (n = 1) mg/kg bw

⁴: Goats treated with 6700 (n = 2) and 4290 (n = 2) mg/kg bw

⁵: Goats treated with 6700 (n = 2) and 4290 (n = 1) mg/kg bw

Table B.6.8-29: Biochemical and haematological measurements of surviving goats (min–max values)

Measurement (units)	Reference value	Control ¹	Definitive signs of intoxication observed (8 goats) ²		Minimal or no signs of intoxication observed (5 goats) ³	
			days 1 and 3	days 7 and 14	days 1 and 3	days 7 and 14
BUN (mg/dL)	10.0–20.0	2.3–21.3	7.6–28.7	3.7–135.6	3.9–13.6	6.0–19.1
SC (mg/dL)	1.0–1.8	0.6–1.7	1.0–1.9	0.8–9.7	0.8–1.4	0.9–1.4
GLU (mg/dL)	50.0–75.0	37–80	60–139	50–90	40–59	29–58
Na (mg/dL)	327–356	311–349	309–337	319–337	323–337	317–337
K (mg/dL)	13.6–26.1	13.4–21.2	12.2–17.6	8.0–19.5	13.9–19.5	15.0–19.4
Ca (mg/dL)	8.9–11.7	8.0–13.4	8.9–11.1	8.7–10.7	9.3–10.1	9.2–10.0
P (mg/dL)	2.9–7.3	4.1–11.9	3.5–10.2	3.8–9.7	4.5–9.3	4.9–8.8
Mg (mg/dL)	2.8–3.6	1.3–3.4	1.8–2.4	1.3–2.4	1.9–2.3	1.9–2.1
SGOT (U/L)	167–513	31–151	34–77	3.7–169	53–81	50–83
LDH (U/L)	123–392	145–385	118–232	141–3.17	170–242	151–238
SACH (U/L)	---	240–496	293–528	209–450	273–386	255–407
ALKP (U/L)	93–387	31–902	58–594	52–542	31–323	30–307
CPK (U/L)	20–42	44–878	58–124	44–180	54–154	44–152
GGT (U/L)	---	12–60	23–43	21–42	15–40	11–30
WBC ⁵	4.0–15.0	5.5–17.5	5.7–19.5	8.0–35.5	7.5–12.5	7.5–20.0
SEGS ⁵	1.6–7.5	1.4–10.4	2.0–14.0	2.0–9.7	2.9–7.2	3.4–16.4
BANDS ⁵	---	0–1.4	0–1.0	0–1.8	0–0.3	0–0.6
LYMPHS ⁵	1.8–9.0	2.4–9.2	2.0–6.7	3.1–8.4	2.9–6.4	2.0–6.3
EOS ⁵	0.1–1.5	0–1.4	0–0.1	0–0.7	0–0.7	0–0.4
MONO ⁵	0.1–0.9	0–0.9	0–0.8	0–0.7	0–0.2	0.1–3.9
BASO ⁵	0–0.2	0–0.8	0–0.3	0–0.6	0–0.5	0–0.4
PCV(%)	19–40	23–47	29–39	27–38	24–32	24–34
HGB (g/dL)	8–16	7.8–16.7	10.4–12.9	9.4–12.4	11.3–12.2	9.8–11.3
RBC ⁶	7–21	12.8–24.2	15.6–23.2	15.7–21.9	18.0–19.0	15.7–17.9
MCV (fL)	15–39	15–21	15.0–19.0	13.0–19.0	14.0–21.0	15.0–21.0
MCH (pg)	5.3–8.4	4.6–7.8	5.1–7.5	5.3–7.0	5.0–6.9	5.2–7.2
MCHC (g/dL)	32–40	28–41	32–38	31–37	33–40	34–41
TSP (g/dL)	6.4–7.0	5.0–8.2	5.9–8.7	5.6–7.7	6.1–7.2	5.9–7.1

¹: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)

²: Goats treated with 6700 (n = 2), 5360 (n = 3) and 4290 (n = 3) mg/kg bw

³: Goats treated with 14000 (n = 3) mg/kg bw

Conclusion

The median lethal dose (LD₅₀) was calculated to be LD₅₀, goat, oral = 5700 mg/kg bw [CI 95 %, 3.73–8.71]

RMS comments: The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is good and it is suitable to provide additional information about acute oral toxicity in a ruminant species. The extent of investigations exceeded that one in acute toxicity studies in laboratory rodents. However, pathology was confined to four of a total of 20 control animals. In addition, assessment was made a bit difficult by the fact that sometimes data obtained in the four control groups were pooled and sometimes reported separately.

The conclusion of the notifier is agreed with. Acute oral toxicity of the isopropylamine salt of glyphosate in goats was low and even lower than with the acid. This finding may confirm that the salt formulations of glyphosate tend to be less toxic than the acid.

B.6.8.3.2 Subacute toxicity in cows

Subacute study with glyphosate isopropylamine salt

Reference: IIA, 5.10/07

Report: [REDACTED] (1987) The subacute toxicity of the isopropylamine salt of glyphosate (MON 0139) in female cattle

[REDACTED]
Data owner: Monsanto
Report No.: VT-82-003
Date: 1987-04-16
TOX9552424

Guidelines: None guideline study

Deviations: Not applicable.

GLP: Yes (*Not checked by RMS. Not compulsory when the study was performed.*)

Acceptability: See RMS comments below.

Dates of experimental work: 1982-02-02 to 1982-05-05

Material and methods

Test material: MON 0139 (isopropylamine salt of glyphosate/
N-phosphonomethyl glycine isopropylamine)

Description: amber liquid

Lot/Batch #: LBRT 08023

Purity: 62.4%, (46.2% glyphosate only)

Stability of test compound: Stable for > 1 year (protocol date: 1980-01-18)

Vehicle and/or positive control: Water

Test animals:

Species: Cow

Strain: not applicable (Brahman cross)

Source: [REDACTED]

Age: not specified

Sex: Female (heifer/nulliparous)

Weight at dosing: 215.6 ± 23.1 kg

Acclimation period: at least 30 days

Diet/Food: During the initial acclimatization (outdoor): bermudagrass hay and commercial grain meal containing not less than 13 % crude protein (Purina ® Commercial Creep Chow ® (Ralston Purina Company, Gonzales, TX, USA). After the initial acclimatization (indoor) the heifers were fed bermudagrass only.

Water: Fresh water

Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study

Environmental conditions: not stated

Study design and methods

Animal assignment and treatment

Dose (based on a preliminary trial with 1000 mg/kg bw)

540, 830, 1290, and 2000 mg/kg bw

Application route: Oral, gavage

Application volume: approx. 500 mL/animal

Fasting time: not stated

Group size: 3/group or 2/group

4 control groups of 2 cows each

Post-treatment observation period: 14 days

Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology

In life dates: not stated

Animal assignment and treatment:

Brahman-cross heifers were treated in a sequential manner with MON 0139 by stomach tube in 7 consecutive daily doses of 540, 830, 1000 (pre-test, n = 2), 1290, and 2000 mg/kg bw (n = 3/group). Initially, the test item was given at 2000 mg/kg bw/day and subsequent dosages were selected based on the observed responses. Control animals were treated with tap water. All animals were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Results and discussion

Mortality

In the preliminary study neither heifer exposed to 1000 mg/kg bw died.

All animals treated with 2000 mg/kg bw MON 0139 died. 1/3 animals treated with 1290 mg/kg bw also died.

The results are summarised in the following table.

Table B.6.8-30: Mortality, survival time and animals used.

Dose (mg/kg bw)	Toxicological result			Onset of death after	Mortality (%)
	Dead animals	Animals with toxic signs	N		
2000	3	3	3	6.2–7.5	100
1290	1	3	3	7.5	33.3
1000*	0	2	2	--	0
830	0	3	3	--	0
540	0	0	3	--	0
0	0	0	8**		0

N: Number of animals

*: Preliminary test

**: All four control groups compiled

The minimum lethal dose (MLD) was 1290 mg/kg bw.

The minimum toxic dose (MTD) was 830 mg/kg bw.

Clinical observations

In the preliminary study, treatment with 1000 mg/kg bw MON 0139 decreased food intake and induced diarrhea by the second day of treatment which continued throughout the seven day treatment period. The signs ceased until the end of the study.

Treatment with 2000, 1290, and 830 mg/kg bw induced diarrhea.

Animals exposed to 2000 mg/kg bw MON 0139 additionally showed nervous system effects including head tremors, convulsions, ataxia, and possible visual impairment and sternal recumbancy.

Table B.6.8-31: Prominent clinical observations in female cows given MON 0139 (numbers of animals affected)

Dose (mg/kg bw)	N	Observations (Number of goats affected)
2000	3	diarrhea (3), decreased feed intake (3), nasal discharge (3), foamy salivation (1), head tremors (3), belligerency (1), whole-body tremors (3), ataxia (3), head pressing (1), kicking at imaginary objects (1), apparent visual impairment (1), convulsions (1), falling (1), depression (1), recumbency (3), increased respiratory effort (2), death (3)
1290	3	diarrhea (3), decreased feed intake (3), depression (3), weakness (2), death (1)
1000	2	diarrhea (2); decreased feed intake (2)
830	3	diarrhea (3), decreased feed intake (3)
540	3	no signs of toxicosis (3)

CFA: cecession of feeding activity

Body weight and food consumption:

Treatment decreased body weight and food intake, as follows. However, the food intake was not precisely measured.

In the preliminary study, treatment with 1000 mg/kg bw MON 0139 decreased food intake.

Treatment with 2000 and 1290 mg/kg bw decreased food intake.

Treatment with 1290 mg/kg bw induced severe weight loss and depression for the first two weeks. While food consumption and fecal consistency returned to normal during the third week after treatment, the animals remained thin and weakened.

The results are summarized in the following table.

Table B.6.8-32: Mean percent body weight changes (from initial body weight) in cows (n) treated with MON 0139

Dose (mg/kg bw/day)	Study day	MON 0139-treated	Controls
2000	6	-1.2 (3)	-1.9 (2)
1290	6	-6.5 ^a (3)	-2.1 (2)
	14	-12.0 ^b (2)	-1.6 (2)
	21	-9.6 (2)	-3.4 (2)
830	6	-5.2 ^a (3)	-1.5 (2)
	14	-4.6 (3)	-0.4 (2)
	21	-2.4 ^a (3)	-5.1 (2)
540	6	0.3 (3)	-0.7 (2)
	14	1.0 (3)	2.7 (2)
	21	0.4 (3)	1.5 (2)

^a: P < 0.08

^b: P < 0.01

Necropsy

Few significant gross lesions were noted in fatally poisoned heifers other than dehydration, loss of weight and signs indicative of gastrointestinal irritation.

Kidney and liver to brain weight ratios were elevated at 2000 and 1290 mg/kg bw/day.

Table B.6.8-33: Mean kidney and liver weight/brain weight ratios

Dose	N	Kidney/brain	Liver/brain
2000	3	1.86 ^a	7.57 ^a
0*	2	1.25	6.45
1290	3	1.49 ^a	5.96
0*	2	0.96	4.81
830	3	1.14 ^a	5.75
0*	2	1.27	6.08
540	3	1.18	6.14
0*	2	1.19	5.92

*: Concurrent control for preceding MON 0139-treated group

^a: P < 0.83

Major gross pathologic findings are summarized in the following table

Table B.6.8-34: Major gross pathologic findings (number)

Dose	Skin	Cardiovascular	Hemic & lymphatic	Hemic & lymphatic	Digestive	Urogenital
2000 (n = 3)	Dermatitis (3)	Aspiration (1) Nodules (1) Pulmonary edema (2)	Epicardial petechiae (3)	Petechiae, capsule of spleen (1)	Errosions, abomasal mucosa (1) Congestion intestine (3) Diarrhea (3) HLBR (3) Distension, gall bladder (1) Congestion liver (1)	HKBR (3)
0* (n = 2)	Dermatitis (2)					Infarct, rt kidney (1)
1290** (n = 3)	Dermatitis (2)	Petechiae, pleura (1)	Epicardial petechiae (1) Hemocyst, heart valve (1)		Scars, capsule of liver (1) Errosions, abomasal mucosa (1) Congestion intestine (1)	HKBR (2)
0* (n = 2)	Dermatitis (2)	Petechiae, trachea mucosa and pleura (1)			Petechiae, duodenum, congestion, peyer's patches (1) Erosion, esophagus (1)	Petechiae, urinary bladder (1)
830 (n = 3)	Dermatitis (1)				Congestion intestine (1) Scars, liver capsule (1)	
0* (n = 2)	Dermatitis (1)		Cogestion, spleen (1)		Petechiae, abomasal mucosa Congestion intestine (2)	Congestion, vulvar mucosa (1)
540 (n = 3)		Subpleural focus (1) Red foci, subpleural (1)			Erosions, esophagus (1) HLBR (1) Congestion intestine (1) Congestion peyer's patches (1) mottling, liver (1)	HKBR (1)
0* (n = 2)	Dermatitis (2)			Lymphoid hyperplasia in pharyngeal nodes (1)	Lipoma (1) Parasitism (1)	

*: Concurrent control for preceding MON 0139-treated group

**: 1/3 bilateral hyphema

NGL: no gross lesions

HLBR: high liver weight to brain weight ratio relative to controls

HKBR: high kidney weight to brain weight ratio relative to controls

No findings for nervous, endocrine, muscular skeletal organs or systems, or the body as a whole

Histopathology

Microscopic examination of kidney from the dead heifer treated with 1290 mg/kg bw/day revealed marked renal tubular vacuolization.

Histologic examination of the abomasum revealed multifocal superficial mucosal erosions, which appeared to be of recent development based on the minimal extent of the associated cellular reaction.

Segmental congestion of the ileum was characterized microscopically by focal necrosis and inflammation of the mucosa overlying Peyer's patches.

The two surviving heifers treated with 1290 mg/kg bw/day each had mild renal tubular vacuolization.

No treatment-related microscopic lesions were observed at dosages of below 1290 mg/kg bw/day or in the 6 of eight control animals that had been subjected to histopathology.

Clinical biochemistry and hematology

Treatment with 1290 and 2000 mg/kg bw/day MON 0139 increased haematocrit, haemoglobin, red blood cells and increased serum levels of total protein, urea nitrogen (BUN), creatinine and serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), and creatine phosphokinase (CPK) activities.

The hematologic alterations were considered to be due to hemo-concentration secondary to fluid shifts resulting from diarrhea.

Elevations in CPK, SGOT, and LDH activities were attributed to muscle damage resulting from convulsions and/or prolonged sternal recumbency.

Slight elevations of BUN and creatinine may have been due to decreased renal perfusion produced by dehydration secondary to diarrhea. However, the presence of histopathological kidney lesions at 1290 mg/kg bw/day and changes in serum electrolyte levels at several dosages suggest that these changes may have been partly due to some renal impairment.

An increase in the number of neutrophils and a decrease in the number of lymphocytes observed at 1290 and 2000 mg/kg bw/day probably represented a response to stress-induced release of corticosteroids.

Remark: Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

The results are summarized in the following tables.

Table B.6.8-35: Days after treatment on which values for clinical laboratory measurements performed on cows treated with MON 0139 were significantly different from concurrent median control values

Measurement	Dose (mg/kg/bw/day)			
	2000	1290 ^a	830	540
	Days after treatment			
BUN	6	2, 6, 7, 8, 14	---	8 ⁻
CREAT	6	7, 8	---	8 ⁻
GLU	---	7	---	---
Na	6	6 ⁻ , 14 ⁻	---	---
K	6 ⁻	6 ⁻ , 8 ⁻ , 14 ⁻	6 ⁻	14 ⁻
Ca	6 ⁻	---	8 ⁻	---
P	6	7, 21 ⁻	2	---

Measurement	Dose (mg/kg/bw/day)			
	2000	1290 ^a	830	540
	Days after treatment			
Mg	6	7, 8	2	---
SGOT	6 [§]	2, 6, 7, 8, 14, 21	---	---
LDH	6 [§]	6, 7	---	---
SACH	---	14 ⁻	21 ⁻	---
ALKP	---	7	---	---
CKN	6	2, 6, 7, 8	---	---
GGT	---	---	---	8, 14, 20
WBC	---	---	---	---
SEGSA	6	---	---	---
BANDSA	6	---	---	---
LYMPHSA	6 ⁻	6 ⁻	---	---
EOSA	2 ^{&} , 6 ^{&}	6 ^{&} , 8 ^{&}	---	14 ⁻ , 21 ⁻
MONOSA	---	---	---	---
BASOA	---	---	---	---
PCV	6	6 [§] , 7, 8	---	---
HBG	6	6, 7, 8	---	---
RBC	6 [§]	6, 7, 8	---	---
MCV	---	---	---	---
MCH	---	---	---	---
MCHC	---	6, 21	---	---
STP	6	6, 7, 8	---	---

^a: represents data from 3 animals on days 2 and 6 and 2 animals on days 8, 14, and 21. Entries for day 7 represent one animal, and the value is outside the range of study-wide control values.

⁻: Medians significantly lower than the concurrent median control value, entries without a symbol represent medians significantly greater than the concurrent median control values

[§]: noteworthy non-significant increase

[&]: noteworthy non-significant decrease

Conclusion

The minimum lethal dose (MLD) was MLD, cow, oral = 1290 mg/kg bw.

The minimum toxic dose (MTD) was MTD, cow, oral = 830 mg/kg bw.

RMS comments: The study is considered acceptable despite the low number of animals per dose group. However, studies of this type are usually not required in Europe for active ingredients in plant protection products and no guideline exists. The quality of the study is good and it is suitable to provide additional information about subacute oral toxicity in a ruminant species. The conclusion of the notifier is agreed with but the "MTD" should be rather considered the LOAEL to make it more comparable to routine toxicological studies and to avoid misunderstandings because "MTD" is often read as "maximum tolerated dose". The low dose of 540 mg/kg bw/day may be considered the NOAEL in this study. Subacute oral toxicity of the isopropylamine salt of glyphosate in young cattle (heifers) was low but signs and findings were more pronounced than in subacute studies (of longer duration) with glyphosate acid in rats and dogs.

Subacute study with a plant protection product

Reference: IIA, 5.10/08

Report: [REDACTED] (1987) The subacute toxicity of Roundup ® herbicide (MON-2139) in female cattle

Data owner: Monsanto

Report No.: VT-82-002

Date: 1987-03-23

ASB2010-8131

Guidelines: Non-guideline study

Deviations: Not applicable.

GLP: Yes (*Not checked by RMS. Not compulsory when the study was performed.*)

Acceptability: See RMS comments below.

Remark (Notifier): The animals were of different hormonal status (observed pregnancy) and partly infested with parasites. This heterogenicity of the study population combined with the small number of animals tested renders in particular the relevance of the clinical biochemistry and hematology findings questionable.

(Comment by RMS: It must be clarified that, according to the original study report, only in one control animal some evidence of parasitic infestation was obtained, based on eosinophilic perivascular infiltrates in the liver. In contrast, many animals on study were suffering from trichophytosis, i.e., a mycotic dermatitis.)

Dates of experimental work: 1982-02-02 to 1982-05-05

Material and methods

Test material: MON 2139 (Roundup ® formulation, N-phosphonomethyl glycine isopropylamine)

Description: amber liquid

Lot/Batch #: LBRP 01002

Purity: 41.1%, (N-phosphonomethyl glycine isopropylamine, 30.5% glyphosate only)

Stability of test compound: Stable for > 1 year (protocol date: 1980-01-18)

Vehicle and/or positive control: Water

Test animals: Species: Cow

Strain: not applicable (Brahman cross)

Source: [REDACTED]

Age: not specified

Sex: Female (heifer/nulliparous)

Weight at dosing: 211.6 ± 21.5 kg

Acclimation period: at least 30 days

Diet/Food: During the initial acclimatization (outdoor): bermudagrass hay and commercial grain meal containing not less than 13% crude protein (Purina ® Commercial Creep Chow ® (Ralston Purina Company, Gonzales, TX, USA). After the initial acclimatization (indoor) the heifers were fed bermudagrass only.

Water: Fresh water

Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study

Environmental conditions: not stated

Study design and methods

Animal assignment and treatment

Dose (based on a preliminary trial with 1000 mg/kg bw)

400, 500, 630, and 790 mg/kg bw

4 control groups included

Application route: oral, rumen intubation

Application volume: approx. 500 mL/animal

Fasting time: not stated

Group size: 3/group or 2/group

Post-treatment observation period: 14 days

Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology

In life dates: not stated

Animal assignment and treatment:

Brahman-cross heifers were treated in a sequential manner with MON 2139 by intubation in 7 consecutive daily doses of 400, 500, 630, 790 (n = 3/group), and 1000 mg/kg bw (n = 2/group, preliminary test). Initially, the test item was given at 1000 mg/kg/bw/day and subsequent dosages were selected based on the observed responses. Control animals (n = 2/group) were treated with fresh water. All animals were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment.

Body weights were determined prior to dosing and for surviving animals on post-dosing days 6, 14, and 21. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 2, 6, 8, 14, and 21. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Results and discussion

Mortality

In the preliminary study both heifers exposed to 1000 mg/kg bw died.

1/3 animals treated with 790 mg/kg bw MON 2139 died.

The results are summarized in the following table.

Table B.6.8-36: Mortality, survival time and animals used.

Dose (mg/kg bw)	Toxicological result			Onset of death after	Mortality (%)
	Dead animals	Animals with toxic signs	N		
1000*	2	2	2	3.3–6-3 days	100
790	1	3	3	8.8 days	33.3
630	0	3	3	---	0
500	0	2	3	---	0
400	0	0	3	---	0
0	0	0	8**	---	0

N: Number of animals

*: Preliminary test

**: Control groups compiled

The minimum lethal dose (MLD) was MLD, cow, oral = 790 mg/kg bw.

The minimum toxic dose (MTD) was MTD, cow, oral = 500 mg/kg bw.

The no effect level (NOEL) was NOEL, cow, oral = 400 mg/kg bw.

Clinical observations

In the preliminary study, treatment with 1000 mg/kg bw MON 2139 induced severe watery diarrhea, cessation of feed intake, and, prior to death, labored respiratory movements.

Treatment with 790 mg/kg bw decreased food intake and induced diarrhea. The two surviving animals had normal stool by day 16 and were eating normally by day 20.

Treatment with 630 mg/kg bw decreased food intake and induced diarrhea.

Treatment with 500 mg/kg bw induced only diarrhea while treatment with 400 mg/kg bw had no effect.

Table B.6.8-37: Prominent clinical observations in female cows given MON 2139 (numbers of animals affected)

Dose (mg/kg bw)	N	Observations (Number of goats affected)
1000*	2	decreased feed intake (2), diarrhea (2), depression (1), expulsion of rumen ingest through the nose (1), labored breathing (2), death (2)
790	3	decreased feed intake (3), diarrhea (2), labored breathing (1), death (1)
630	3	decreased feed intake (3), diarrhea (2), soft feces (1)
500	3	soft feces (2), no signs of toxicosis (1)
400	3	no signs of toxicosis (3)

*: Preliminary test

Body weight and food consumption

Treatment 630 and 400 mg/kg bw MON-2139 decreased body weight on day 14 and treatment with 500 mg/kg bw increased body weight on day 6 compared to controls. However, statistical significance was not reached.

The results are summarised in the following table.

Table B.6.8-38: Mean percent body weight changes (from initial body weight) in cows (n) treated with MON 2139

Dose (mg/kg bw/day)	Study day	MON 2139-treated	Controls
790	6	-1.3 (3)	-2.1 (2)
	14	-7.6 (2)	-1.6 (2)
	21	-9.0 (2)	-3.4 (2)
630	6	1.4 (3)	-0.7 (2)
	14	-0.5 ^a (3)	2.7 (2)
	21	-0.5 (3)	1.5 (2)
500	6	5.3 ^b (3)	-1.9 (2)
	14	-0.2 (3)	-0.8 (1)
	21	1.9 (3)	1.2 (1)
400	6	-1.2 (3)	-1.4 (2)
	14	-2.5 ^a (3)	-0.4 (2)
	21	-0.3 (3)	5.1 (2)

^a: approaches being significantly less than control mean ($0.05 < P < 0.10$)

^b: approaches being significantly greater than control mean ($0.05 < P < 0.1$)

Necropsy

No consistent gross post-mortem lesions were observed in any of the MON 2139- treated or control animals.

The only treatment-related finding was aspiration pneumonia in the 790 mg/kg bw-treated animal that died. One of the animals in the preliminary study also exhibited gross lung lesions consistent with broncho-pneumonia.

Mean kidney and liver weights are presented in the following table.

Table B.6.8-39: Mean kidney and liver weight/brain weight ratios after treatment with MON 2139

Dose (mg/kg bw)	N	Kidney/brain	Liver/brain
790	3	1.44 ^a	5.80 ^a
0*	2	0.96	4.81
630	3	1.28	5.91
0*	2	1.19	5.92
500	3	1.35 ^a	5.88
0*	2	1.25	6.45
400	3	1.31	5.53
0*	2	1.27	6.08

*: Concurrent control for preceding MON 2139-treated group

^a: P < 0.83

Major gross pathologic findings are summarized in the following table.

Table B.6.8-40: Major gross pathologic findings (number)

Body as a whole	Skin	Respiratory	Cardiovascular	Hemic & Lymphatic	Digestive	Urogenital
790 mg/kg bw (n = 3)						
Autolysis (1/3) Weight loss (1/3)	Dermatitis (3/3)	Congestion, tracheal mucosa, broncho-pneumonia (1/3)	Petechiae, epicardial (1/3) Pallor heart (1/3)	Congestions, bronchial lymph nodes (1/3)	Abomastitis, congestion colon (1/3) Discoloration, rumen mucosa (1/3) Hyperkeratosis, tongue (1/3) erosions esophagus (1/3) darkening, rumen (1/3) petechiae, abomasum 1/3)	Congestion, kidneys (1/3) Fetus (1/3) HKBR (2/3)
Concurrent control for 790 mg/kg bw (n = 2)						
	Dermatitis (2/2)	Petechia, trachea & pleura (1/2)			Erosion, esophagus (1/2) Petechia, tuodenum (1/2) congestions, Peyer's patches (1/2)	

Body as a whole	Skin	Respiratory	Cardiovascular	Hemic & Lymphatic	Digestive	Urogenital
630mg/kg bw (n = 3)						
	Dermatitis (1/3)			Lymphoid hyperplasia, pharyngeal nodes (1/3) Slight congestion, spleen (1/3)	Focal fibrosis, liver (1/2) Parasitism (2/3)	HKBR (1/3)
Concurrent control for 630mg/kg bw (n = 2)						
	Dermatitis (1/2)			Lymphoid hyperplasia, pharyngeal nodes (mild) (1/2)	Parasitism (1/2) Lipoma (1/2)	
500 mg/kg bw (n = 3)						
Weight loss (3/3)	Dermatitis (3/3)	Petechiae, tracheal mucosa (1/3)		Congestion (1/3)	Erosion, esophagus (1/3) Petechiae, gall bladder (1/3)	HKBR (3/3)
Concurrent control for 500 mg/kg bw (n = 2)						
Weight loss (2/2)	Dermatitis (2/2)				Infarct, right kidney (1/2)	
400 mg/kg bw (n = 3) ^a						
	Dermatitis (1/3)	Adhesions, pleura (1/2)			Congestion, ileum (1/3) Parasitism (1/3)	HKBR (2/3)
Concurrent control for 500 mg/kg bw (n = 2)						
	Dermatitis (1/2)				Congestion ileum & cecum (1/2) Congestion, illeal mucosa (1/2) Petechia, abomasal mucosa (1/2)	Congestion, vulvar mucosa (1/3)

^a: 1/3 animals showed melanosis in the adrenal capsule and 1/3 animals showed hydrocephalus

NGL: no gross lesions

HKBR: high kidney weight to brain weight ratio relative to controls

No findings for special senses or the skeletal muscle.

Histopathology

Microscopic tissue examination confirmed the presence of aspiration pneumonia in the 790 mg/kg bw-treated animal that died. One of the 790 mg/kg bw-treated animals was found to be carrying a live, apparently normal fetus when sacrificed at study termination.

There were no other treatment-related microscopic findings.

Clinical biochemistry and hematology

Treatment with 790 mg/kg bw/day MON 2139 increased serum urea nitrogen (BUN) and electrolytes, serum enzyme activities (SECT, GGT), hemoglobin concentration, and red blood cell counts.

Significant changes in group median and individual clinical laboratory values at lower dose levels were not considered to be treatment-related since no dose-response relationship was evident.

The results are summarized in the following tables.

Table B.6.8-41: Days after treatment on which values for clinical laboratory measurements performed on cows treated with MON 2139 were significantly different from concurrent median control values

Measurement	Dose (mg/kg/bw/day)			
	790	630	500	400
	Days after treatment			
BUN	8, 14	---	---	21 ^a
CREAT	---	---	---	2 ^a , 6 ^a , 8 ^a , 14 ^a
GLU	21 ^a	---	2 ^a , 8 ^a	---
Na	14 ^a	---	---	---
K	14 ^a , 21 ^a	---	---	---
Ca	---	---	---	---
P	14 ^a , 21 ^a	6 ^a	---	---
Mg	---	---	---	---
SGOT	14, 21	---	---	---
LDH	14 ^a	---	8 ^a , 14 ^a	---
SACH	---	---	---	---
ALKP	8 ^a , 14 ^a , 21 ^a	---	2 ^a , 6 ^a , 8 ^a	---
CKN	---	---	---	---
GGT	21	---	---	---
WBC	---	---	---	---
SEGSA	---	---	---	---
BANDSA	8	---	---	6, 14
LYMPHSA	---	---	---	---
EOSA	14 ^a	---	---	---
MONOSA	---	---	---	---
BASOA	---	---	---	---
PCV	---	---	---	---
HBG	6, 8	---	---	---
RBC	6, 8	---	---	---
MCV	---	---	---	---
MCH	---	---	---	---
MCHC	6, 14	2	---	---
STP	---	---	8 ^a , 14 ^a	---

^a: medians significantly lower than the concurrent median control value; entries without this symbol represent medians significantly greater than the concurrent median control values.

Conclusion

The minimum lethal dose (MLD) was MLD, cow, oral = 790 mg/kg bw.

The minimum toxic dose (MTD) was MTD, cow, oral = 500 mg/kg bw.

The no effect level (NOEL) was NOEL, cow, oral = 400 mg/kg bw.

RMS comments: The study is considered supplementary because of the uncertainty with regard to parallel parasitic infestation and dermal mycosis that might have weakened the animals. Nonetheless, the study is suitable to provide additional information about subacute oral toxicity of a Roundup formulation in a ruminant species and for comparison with effects of the isopropylamine salt. The conclusion of the notifier is agreed with but the "MTD" should be rather considered the LOAEL to make it more comparable to routine toxicological studies and to avoid misunderstandings because "MTD" is often read as "maximum tolerated

dose". The low dose of 400 mg/kg bw/day was the NOAEL in this study. Toxic signs and mortality occurred at dose levels that were lower than in the similar study with the isopropylamine salt of glyphosate suggesting a higher toxicity of MON 2139. This finding is in line with information from other sources pointing to a higher toxicity of certain formulations as compared to glyphosate and its salts.

B.6.8.3.3 Published data

Krüger *et al.* (2013, ASB2013-11599) reported the abundance of glyphosate in the urine of a total of 240 cows from Denmark. From each of eight dairy farms, the same number of 30 cows (15 fresh calving, 15 high yielding cows, *i.e.*, at the top level of milk production) was selected. All these 240 cows excreted glyphosate in the urine, however, at very different concentrations. Urine samples were diluted 1:20 with distilled water and tested for glyphosate by means of an ELISA kit (Abraxis, USA). It is stated in the paper that validation of test results had been done by a comparison with GC-MS which is considered a more suitable method. It was mentioned that the correlation coefficient between the two methods was 0.96 and, thus, sufficiently high but this validation data was not shown in the paper. However, for further interpretation of the results, it is assumed that the method was in fact valid and that the measured values were reliable.

It is worth mentioning that the cows (breed not given) were 4 to 7 years old and had an average body weight between 550 – 600 kg and that the total number of cows in the farms ranged from 140 to 400 animals. The average daily milk yield in the different farms ranged from 8.6 to 11.2 kg, *i.e.*, was representative for countries with modern and efficient agriculture.

Mean urinary glyphosate concentrations differed very much among the eight farms, ranging from 10 ng/mL up to 103.3 ng/mL. It is a reasonable assumption that urinary excretion of glyphosate was due to dietary exposure and, thus, detection of glyphosate in the urine of cattle is not surprising. Residues of glyphosate may occur in feedstuffs for ruminants and, so far the MRLs are not exceeded, are allowed and of no concern. (Therefore, the word "contamination" as used by the authors is not correct and somehow misleading.) The reason for the remarkable differences between the farms is unknown but most probably the diet was different, including a high variability in glyphosate residues. It is one of the main deficiencies of the publication that no details on feeding regime have been given and that the diets were not analysed for glyphosate content.

It is interesting to note that the mean urinary concentrations in cows exceeded the mean value in human urines of 0.2 µg/L as found by Hoppe (2013, ASB2013-8037) by at least 50 (up to more than 500) times suggesting higher residues of glyphosate in cattle rations than in human diet.

The maximum mean value of ca 103 µg/L can be used to calculate a systemic dose to which the cows in that farm had been exposed to since glyphosate does not accumulate but is rapidly excreted. The study authors have estimated a maximal glyphosate excretion via urine of 3.1 mg/day. If an oral absorption rate of only 20 % is assumed instead of 30 % as used by the study authors (for justification, see B.6.1), the maximum daily oral intake should have been in the magnitude of 15 – 16 mg. This dose might be compared either to the ADI (even though it is not clear if a reference dose established for humans is *per se* also applicable to farm animals) or to the NOAELs in the subacute studies in cattle mentioned above.

If the first approach is taken, the use of the proposed ADI of 0.5 mg/kg body weight for glyphosate (see Vol. 1, B.6.12) would result in a tolerable intake of 225 mg for a cow of 550 kg body weight. This amount is by about 14 – 15 times higher than the expected maximal exposure of the Danish cows on study or, in other words, the systemic dose in the cow with highest urinary excretion of glyphosate would account for not more than about 7 % of the ADI.

Using the second approach, the maximum expected systemic dose was compared to the NOAELs in subacute studies with either the isopropylamine salt of glyphosate (540 mg/kg bw/day according to Rowe et al., 1987, TOX9552424) or a Roundup formulation (400 mg/kg bw/day according to Rowe et al., 1987, ASB2010-8131). For a cow of 550 kg, the latter NOAEL would correspond to a daily intake of 220 g. In the study description (see above), a glyphosate content of 30.5% is mentioned. Thus, a daily glyphosate intake of 67 g can be calculated. If this amount of 67000 mg is compared to the expected maximum systemic exposure of 15 or 16 mg per day in the study in Danish cows, a margin of safety of about 1:4200 would result. , Based on these considerations, an impairment of animal health in these cows is very unlikely.

In contrast, the authors reported increased activities of the enzymes creatine kinase (CK), glutamate oxaloacetate transaminase (GOT, synonymous to ASAT), and glutamate dehydrogenase (GLDH), but also changes in cholesterol levels and an increase in blood urea concentrations when compared to (mostly not precisely mentioned) reference values of unknown origin. They postulated a relationship with the detection of glyphosate in urine interpreting the alterations on laboratory parameters as indicative for liver damage or nephrotoxicity.

Leaving aside the serious methodical deficiencies of the study (*e.g.*, the absence of a control group with no glyphosate residues in the urine), there is no evidence that the altered clinical chemistry parameters in cows were indicative of any health deterioration. There could be many different reasons of such alterations and, taking into account the very low glyphosate concentrations (see above), exposure to glyphosate is not a likely one. Moreover, the statistical correlation between glyphosate excretion in urine and changed clinical chemistry parameters as claimed by the authors was in fact rather poor. (Thus, for the comparison of glyphosate with creatine kinase, an R value of 0.135 was considered in the article as indicative of “positive correlation” which in fact is not the case. In contrast, higher R values of up to 0.809 were obtained when two different clinical chemistry parameters such as zinc and cobalt concentrations were measured and may indicate some correlation between them but apparently not with glyphosate excretion. Unfortunately, due to the way of reporting, the reader may be misled here to assume correlation with glyphosate.)

Since, in parallel, the authors claimed very low levels of several trace elements such as cobalt or manganese, a possible chelating mode of action of glyphosate was suspected. However, these considerations appear purely speculative, in particular against the background of the very low exposure. Again, statistical correlation was rather weak. Indeed, the almost complete absence of the two elements in cattle from all farms rather points to either an analytical problem or to a deficiency in the diet.

Thus, to conclude, the urinary levels in Danish cows might well reflect the abundance of glyphosate residues in their feed. The systemic exposure that may be calculated is very low and no health concern is anticipated. Final conclusion on the clinical chemistry findings is not possible but, on one hand, it seems not proven that they were adverse and, on the other, it is very unlikely that they might be related to glyphosate.

A number of papers has been published recently in which a possible causal link of glyphosate exposure and subsequent *Clostridium botulinum* (*C. botulinum*) overgrowth with a new disease in cattle is suggested. The scientific background of this assumption is the herbicidal mode of action of glyphosate. In plants, the enzyme 5-enolpyruvylshikimate acid-3-phosphate synthase (EPSP synthase) is inhibited resulting in a lack of formation of aromatic amino acids by the shikimate pathway that is common in the plant kingdom but not does not occur in animals. However, this pathway is operative in most bacteria and yeast and many protozoan species. Thus, an impact of glyphosate on microflora, e.g., in the intestines is at least conceivable.

Rodloff and Krüger (2012, ASB2013-13311) hypothesised that an emerging new disease in cattle but also symptoms in a small number of farmers might be caused by *Clostridium botulinum*. This animal disease of so far unknown etiology and pathogenesis was reported to have occurred from the late 1990ies onwards in cattle mainly from some parts of Germany but, according to the authors, cases had been observed also in France, the Netherlands, and the U.K. even though references were not given. Clinical signs in cattle are predominately seen in the perinatal period and comprise indigestion with alternating constipation and diarrhea, apathy, ataxia, paralysis, retracted abdomen, breathing difficulties, a decrease in milk yield, and death. In some farmers taking care of affected herds, symptoms such as dizziness, weakness, fatigue, blurred vision, nausea, and difficulties to speak, to swallow and to breathe have been occasionally reported.

The authors suggest a causal link to *Clostridium botulinum* because these signs and symptoms appear similar to what is known from the rarely occurring cases of botulism in animals and man that are not food-borne (i.e., not caused by acute intoxication with the bacterial toxin). By means of an ELISA (no details given) they have detected *Clostridium botulinum* neurotoxin (BoNT) in fecal samples (in total in 16 out of 33) obtained from cattle on six German farms where the suspicious clinical signs or even fatalities had been noted but not in a total of 10 samples from two farms without any evidence of this disease. Toxin types were different (A, B, C, D, and E, in various combinations). Health state of the donor animals was not reported. In addition, neurotoxin of different types was found in various organs such as the rumen and the liver in 15 sick cows after slaughter. However, it is not known how many cows were examined in total *post mortem* and by which method and if they belonged to the group of which fecal samples had been taken before.

In man, 16 out of 77 fecal samples were positive for BoNT that were taken from humans who were reported to have been in close contact with diseased cows. It is not clear if one or more samples were obtained from the same person and, thus, the total number of humans under investigation is not known. Again, health status of the involved people was not reported. With regard to neurotoxin types, mainly type E had been found.

Unfortunately, these numbers do not completely match with a table in the paper in which 12 BoNT-positive human fecal samples in a total number of 33 and 29 BoNT-positive bovine fecal samples out of 118 under examination were mentioned to be obtained from 7 “selected” German cattle farms.

Conclusion by RMS:

This paper does not contribute anything to risk assessment of glyphosate and, in fact, this herbicidal active ingredient, was not mentioned therein. However, it is important for understanding of subsequent publications and, therefore, is referred to in this RAR.

In itself, this paper is adequate to suggest a scientific hypothesis, based on some data that might require further research. However, no causal relationship between a new disease in cattle and *C. botulinum* has been established. *C. botulinum* neurotoxin could not be

quantified. Qualitative detection of the neurotoxin might be also a random co-incidence. The publication is flawed by many reporting deficiencies. In particular, the method by which the neurotoxin was detected and its different types were distinguished, is not described.

In a similar paper, Krüger *et al.* (2012, ASB2013-13312) reported the abundance of (different types) of the micro-organism *C. botulinum* itself in 44 out of 196 bovine fecal samples (22.5%) and in 17 out of 77 human fecal samples (22%) but also in silages ($9 / 21 = 47\%$), concentrate feed specimens ($4 / 14 = 28.6\%$), and in all 7 tested house dust specimens from a total of 41 dairy farms in the German federal state of Schleswig-Holstein. This finding was based on the immunological detection of antigens of *C. botulinum* by an ELISA technique using polyclonal antibodies. All the cattle had been reported to have shown clinical signs as described above. In addition, in four of the involved humans, symptoms were claimed to have occurred but without further specification or medical confirmation.

Conclusion by RMS:

Again, this paper, in principle, is not relevant for risk assessment of glyphosate but it is mentioned therein that ingested spores might germinate in the intestinal tract if protective indigenous bacterial flora is lacking (as observed in cases of infant botulism) or altered leading to the glyphosate hypothesis explained below. Therefore, it is mentioned here despite its many reporting deficiencies. The findings rather point to ubiquitous occurrence of *C. botulinum* but are not suitable to prove a causal relationship of its abundance to clinical signs or symptoms.

By the same group, it was published that different bacteria such as *Enterococcus faecalis*, *Enterococcus faecium* or *Bacillus badius* were able to inhibit growth of *C. botulinum* and/or the production of its neurotoxins *in vitro* whereas other bacterial species did not exhibit such an effect (Shehata *et al.*, ASB2013-8529). Subsequently, Krüger *et al.* (2013, ASB2013-8527) reported that glyphosate (analytical grade) and the herbicide Roundup UltraMax® containing 450 glyphosate/mL was able to suppress this antagonizing effect of *Enterococcus* species on *C. botulinum* *in vitro*. What they actually observed was a growth inhibition of both *C. botulinum* (and a reduction in neurotoxin type B production) and *Enterococcus faecalis* by glyphosate and Roundup herbicide but at different concentrations. While growth of *E. faecalis* was completely inhibited by 0.1 mg glyphosate or Roundup/mL, the same effect on *C. botulinum* was seen only at a concentration of 1 mg Roundup/mL or of 10 mg glyphosate/mL.

Conclusion by RMS:

This data suggests a different susceptibility of *E. faecalis* and *C. botulinum* to cytotoxic effects of glyphosate and a glyphosate-based herbicide *in vitro*. With regard to *C. botulinum*, the Roundup UltraMax formulation was more toxic than the active ingredient confirming similar evidence from various fields of toxicological testing. No conclusions can be drawn if similar effects might occur *in vivo* because the situation in, e.g., the GITs of ruminants or monogastric animals is different with hundreds or thousands of microbial species co-existing. In any case, even the lowest tested concentration of 0.1 mg/mL appears extremely high if a (maximum) systemic dose of 15 or 16 mg per cow is assumed to result from feeding the animals with a ration containing glyphosate residues (based on [REDACTED], 2013, ASB2013-11599, see above). Thus, the possible impact of glyphosate (herbicides) on bacteria due to inhibition of the enzyme EPSP was somehow confirmed *in vitro* but there is no health concern and no impact on realistic risk assessment.

A different toxicity of Roundup UltraMax® to various microbial species was also observed by Shehata *et al.* (2013, online published in 2012 yet, ASB-2012-16301) who measured the effect of different concentrations on 23 bacterial species and strains mostly of chicken origin and also on sporulated *Eimeria tenella* (i.e., a protozoon in poultry) oocytes *in vitro*. It is not clear whether the mentioned concentrations ranging from 0.075 mg to up to 5 (bacteria) or 1.2 (*Eimeria tenella*) mg per mL are related to the herbicide formulation or had been adjusted to the active ingredient. In general, the authors found lower minimum inhibitory concentrations (0.075 – 0.6 mg/mL) for beneficial bacteria whereas, in contrast, some pathogenic germs such as *Clostridium perfringens* or several *Salmonella* species appeared much less sensitive with growth inhibition seen only at the highest tested concentration of 5 mg/mL. With regard to *Eimeria tenella*, the threshold for an effects was around 0.3 mg/mL with a clear effect to be seen at 0.6 mg/mL.

Conclusion by RMS:

Different cytotoxicity of a glyphosate-based herbicide to micro-organisms was confirmed once more and might be due to either the active ingredient or, e.g., a surfactant. (It is not known whether a [REDACTED] surfactant was contained.) However, antibiotic activity of the herbicide (expressed in the minimum inhibitory concentrations) was lower than that of known antibiotics that are used in veterinary medicine. Even the lowest effect concentrations in this study were by far higher than the expected glyphosate concentrations in poultry feed (GTF, 2013; ASB2013-11007) and, thus, must be considered unrealistically high. Furthermore, in vitro exposure of selected individual species and strains to a herbicide might be not a good model for complex interactions in GIT of poultry when residues are ingested.

To conclude, a link of glyphosate residues in ruminants diet to a new disease in cattle has not been established and is not likely. Furthermore, there is no convincing proof that clinical signs in cattle (of which the occurrence cannot be doubted) were indeed caused by *C. botulinum* or its toxins. However, because of the growing public concern about this cattle disease especially in Germany and because an effect on micro-organisms due to EPSP inhibition cannot be excluded, the German Federal Institute for Risk Assessment (BfR) has commissioned a study with a glyphosate-based herbicide (containing a [REDACTED] surfactant) in an artificial rumen system (RUSITEC) to investigate whether (1) quantitative composition of ruminal microflora might be compromised and (2) there is evidence of *C. botulinum* overgrowth. Unfortunately, results of this project were not available when this RAR was finalised (October, 2013) but it is hoped that they will be published in 2014.

B.6.8.4 Further published data (released since 2000)

Introduction by RMS:

A large number of studies on toxicity of glyphosate and its formulations was published since 2000. Most of these studies are presented in the chapters on genotoxicity, carcinogenicity, reproductive toxicity and neurotoxicity of this report because they are discussed there in context with these endpoints. However, some additional studies are presented below that could not be allocated to these endpoints.

Author(s)	Year	Study title
Robert Bellé, Ronan Le Bouffant, Julia Morales, Bertrand Cosson, Patrick Cormier et Odile Mulner-Lorillon	2007	L'embryon d'oursin, le point de surveillance de l'ADN endommagé de la division cellulaire et les mécanismes à l'origine de la cancérisation. Journal de la Société de Biologie, Volume : 201, Number: 3, Pages: 317-327 ASB2012-11560

Abstract*

Sea urchin embryo, DNA-damaged cell cycle checkpoint and the mechanisms initiating cancer development (translation from original article)

Cell division is an essential process for heredity, maintenance and evolution of the whole living kingdom. Sea urchin early development represents an excellent experimental model for the analysis of cell cycle checkpoint mechanisms since embryonic cells contain a functional DNA-damage checkpoint and since the whole sea urchin genome is sequenced. The DNA-damaged checkpoint is responsible for an arrest in the cell cycle when DNA is damaged or incorrectly replicated, for activation of the DNA repair mechanism, and for commitment to cell death by apoptosis in the case of failure to repair. New insights in cancer biology lead to two fundamental concepts about the very first origin of cancerogenesis. Cancers result from dysfunction of DNA-damaged checkpoints and cancers appear as a result of normal stem cell (NCS) transformation into a cancer stem cell (CSC). The second aspect suggests a new definition of "cancer", since CSC can be detected well before any clinical evidence. Since early development starts from the zygote, which is a primary stem cell, sea urchin early development allows analysis of the early steps of the cancerization process. Although sea urchins do not develop cancers, the model is alternative and complementary to stem cells which are not easy to isolate, do not divide in a short time and do not divide synchronously. In the field of toxicology and incidence on human health, the sea urchin experimental model allows assessment of cancer risk from single or combined molecules long before any epidemiologic evidence is available. Sea urchin embryos were used to test the worldwide used pesticide Roundup that contains glyphosate as the active herbicide agent; it was shown to activate the DNA-damage checkpoint of the first cell cycle of development. The model therefore allows considerable increase in risk evaluation of new products in the field of cancer and offers a tool for the discovery of molecular markers for early diagnostic in cancer biology. Prevention and early diagnosis are two decisive elements of human cancer therapy.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not assignable

Comment:

Documentation insufficient for evaluation.

The publication overview provides information on the general application of the sea urchin embryo model for the prediction of "cancerogenicity". Only a short reference to another study with a glyphosate-containing herbicide is given. Details of the glyphosate product are not provided. Common surfactants have previously shown the same effects in this model. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.

Relevance of study:

Not relevant (Prevention of cell cycle transition was determined

for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants.)

Klimisch code:

4

Author(s)	Year	Study title
Marc J., Mulner-Lorillon, O., Boulben, S, Hureau, D. Durand, G., Belle, R.	2002	Pesticide Roundup Provokes Cell Division Dysfunction at the level of CDK1/Cyclin B Activation.. Chem. Res. Toxicol. 2002, 15, 326-331 ASB2013-9838

Abstract*

To assess human health risk from environmental chemicals, we have studied the effect on cell cycle regulation of the widely used glyphosate-containing pesticide Roundup. As a model system we have used sea urchin embryonic first divisions following fertilization., which are appropriate for the study of universal cell cycle regulation without interference with transcription. We show that 0.8 % Roundup (containing 8 mM glyphosate) induces a delay in the kinetic of the first cell cleavage of sea urchin embryos. The delay is dependent on the concentration of Roundup. The delay in the cell cycle could be induced using increasing glyphosate concentrations (1-10 mM) in the presence of a subthreshold concentration of Roundup 0.2 %, while glyphosate alone was ineffective, thus indicating synergy between glyphosate and Roundup formulation products. The effect of Roundup was not lethal and involved a delay in entry into M-phase of the cell cycle, we analysed CDK1/cyclin B activation during the first division of early development. Roundup delayed the activation of CDK1/cyclin B *in vivo*. Roundup inhibited also the global protein synthetic rate without preventing the accumulation of cyclin B. In summary, Roundup affects cell cycle regulation by delaying activation of the CDK1/cyclin B complex, by synergic effect of glyphosate and formulation products. Considering the universality among species of the CDK1/cyclin B regulator, our results question the safety of glyphosate and Roundup on human health.

* Quoted from article

Author(s)	Year	Study title
Marc J., Mulner-Lorillon, O., Durand, G., Belle, R.	2003	Embryonic cell cycle for risk assessment of pesticides at the molecular level. Environmental Chemistry Letters Volume: 1, Number: 1, Pages: 8-12 ASB2009-9013

Abstract*

Cell cycle mechanisms are highly conserved from unicellular eukaryotes to complex metazoans including humans. Abnormalities in the regulation of the cell cycle result in death or diseases such as cancer. Early development of sea urchin has proved to be a powerful model for cell division studies and offers the opportunity to study synchronous cell divisions in the absence of transcriptional control. We have analyzed pesticide induced dysfunctions in the first cell division following fertilization in sea urchin embryos, using Roundup, a widely used pesticide formulation containing isopropylamine glyphosate as the active substance. The pesticide induced cell cycle dysfunction by preventing the *in vivo* activation of the universal

cell cycle regulator CDK1/cyclin B. We further show that synthesis of the regulator protein, cyclin B, as well as its association to the catalytic protein, CDK1, were not affected by the pesticide. Therefore, our results suggest that the pollutant impedes the processing of the CDK1/cyclin B complex, which is required in its physiological activation. Our studies demonstrate the relevance of sea urchin embryonic cells as a sensitive model to assess pesticide toxicity at the level of the universal cell cycle checkpoints.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Mechanistic study. Outcome with little additional information compared to the authors' previously published work. Non-standard, non-guideline. Commonly used surfactants have previously shown the same effects in this model.
Relevance of study:	Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using <i>in vitro</i> systems.)
Klimisch code:	3

Author(s)	Year	Study title
Marc, J. Mulner-Lorillon, O. Belle, R.	2004	Glyphosate-based pesticides affect cell cycle regulation Biology of the Cell Volume: 96, Pages: 245-249, ASB2009-9014

Abstract*

Cell-cycle dys-regulation is a hallmark of tumor cells and human cancers. Failure in the cell-cycle checkpoints leads to genomic instability and subsequent development of cancers from the initial affected cell. A worldwide used product Roundup 3plus, based on glyphosate as the active herbicide, was suggested to be of human health concern since it induced cell cycle dysfunction as judged from analysis of the first cell division of sea urchin embryos, a recognized model for cell cycle studies. Several glyphosate-based pesticides from different manufacturers were assayed in comparison with Roundup 3plus for their ability to interfere with the cell cycle regulation. All the tested products, Amega, Cargly, Cosmic, and Roundup Biovert induced cell cycle dysfunction. The threshold concentration for induction of cell cycle dysfunction was evaluated for each product and suggests high risk by inhalation for people in the vicinity of the pesticide handling sprayed at 500 to 4000 times higher dose than the cell-cycle adverse concentration.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not Reliable
Comment:	Non-standard, non-guideline study. Commonly used

Relevance of study:

surfactants have previously shown the same effects in this model.

Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.

Klimisch code:

3

Author(s)	Year	Study title
Marc, J. Belle, R. Morales, J. Cormier, P. Mulner-Lorillon, O.	2004	Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition. Toxicological Sciences, Volume 82, Pages: 436-442 ASB2012-11894

Abstract*

A glyphosate containing pesticide impedes at 10 mM glyphosate the G2/M transition as judged from analysis of the first cell cycle of sea urchin development. We show that formulated glyphosate prevented dephosphorylation of Tyr 15 of the cell cycle regulator CDK1/cyclin B *in vivo*, the end point target of the G2/M cell cycle checkpoint. Formulated glyphosate had no direct effect on the dual specific cdc25 phosphatase activity responsible for Tyr 15 dephosphorylation. At a concentration that efficiently impeded the cell cycle, formulated glyphosate inhibited the synthesis of DNA occurring in S phase of the cell cycle. The extent of the inhibition of DNA synthesis by formulated glyphosate was correlated with the effect on the cell cycle. We conclude that formulated glyphosate's effect on the cell cycle is exerted at the level of the DNA-response checkpoint of S phase. The resulting inhibition of CDK1 cyclin B Tyr 15 dephosphorylation leads to prevention of the G2/M transition and cell cycle progression.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Non-standard, non-guideline study. Commonly used surfactants have previously shown the same effects in this model.

Relevance of study:

Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.

Klimisch code:

3

Additional comments:

Comments of the notifier are submitted on the web site of Monsanto (2006, ASB2013-5455):

http://www.monsanto.com/products/Documents/glyphosate-background-materials/Response_ISIS_apr_06.pdf

The following two recent publications, by Heu *et al.* (2012, ASB2012-11843 and ASB2012-11844) are commented on collectively after the second summary/Klimisch rating, below.

Author(s)	Year	Study title
Heu, C., Berquand, A., Elie-Caille, C., Nicod, L.	2012	Glyphosate-induced stiffening of HaCat keratinocytes, a Peak Force Tapping study on living cells. Journal of Structural Biology Volume: 178, Number: 1 Pages: 1-7 ASB2012-11843

Abstract*

The skin is the first physiological barrier, with a complex constitution, that provides defensive functions against multiple physical and chemical aggressions. Glyphosate is an extensively used herbicide that has been shown to increase the risk of cancer. Moreover there is increasing evidence suggesting that the mechanical phenotype plays an important role in malignant transformation. Atomic force microscopy (AFM) has emerged within the last decade as a powerful tool for providing a nanometer-scale resolution imaging of biological samples. Peak Force Tapping (PFT) is a newly released AFM-based investigation technique allowing extraction of chemical and mechanical properties from a wide range of samples at a relatively high speed and a high resolution. The present work uses the PFT technology to investigate HaCaT keratinocytes, a human epidermal cell line, and offers an original approach to study chemically-induced changes in the cellular mechanical properties under near-physiological conditions. These experiments indicate glyphosate induces cell membrane stiffening, and the appearance of cytoskeleton structures at a subcellular level, for low cytotoxic concentrations whereas cells exposed to IC₅₀ (inhibitory concentration 50 %) treatment exhibit control-like mechanical behavior despite obvious membrane damages. Quercetin, a well-known antioxidant, reverses the glyphosate-induced mechanical phenotype.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline <i>in vitro</i> tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)
Relevance of study:	Not relevant (<i>in vitro</i> data on the effects on an immortalised epidermal cell-line does consider low exposure potential due to <i>stratum corneum</i> protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not in formulated glyphosate based products)
Klimisch code:	3

Author(s)	Year	Study title
Heu, C., Elie-Caille, C., Mougey, V., Launay, S., Nicod, L.	2012	A step further towards glyphosate-induced epidermal cell death: Involvement of mitochondrial and oxidative mechanisms. Environmental Toxicology and Pharmacology Volume: 34, Number: 2, Pages: 144-153 ASB2012-11844

Abstract*

A deregulation of programmed cell death mechanisms in human epidermis leads to skin pathologies. We previously showed that glyphosate, an extensively used herbicide, provoked cytotoxic effects on cultured human keratinocytes, affecting their antioxidant capacities and impairing morphological and functional cell characteristics. The aim of the present study, carried out on the human epidermal cell line HaCaT, was to examine the part of apoptosis plays in the cytotoxic effects of glyphosate and the intracellular mechanisms involved in the apoptotic events. We have conducted different incubation periods to reveal the specific events in glyphosate-induced cell death. We observed an increase in the number of early apoptotic cells at a low cytotoxicity level (15%), and then, a decrease, in favour of late apoptotic and necrotic cell rates for more severe cytotoxicity conditions. At the same time, we showed that the glyphosate-induced mitochondrial membrane potential disruption could be a cause of apoptosis in keratinocyte cultures.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Non-guideline *in vitro* tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)

Relevance of study:

Not relevant (*in vitro* data on the effects on an immortalized epidermal cell-line does consider low exposure potential due to *stratum cornea* protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not contained in formulated glyphosate based products)

Klimisch code:

3

Additional comments:

Glyphosate technical acid evaluated was not reported to be pH adjusted and therefore does not reflect real world exposures to the more neutral pH formulations, which contain glyphosate salts, not glyphosate acid

The pH range of test concentrations (850-1150 mg/L) is very acidic, approximately 1.7-2-2 pH units. Keeping in mind the pH scale is logarithmic, these values are substantially lower than those of viable skin and *in vitro* cell cultures.

Exposure potential to live human epidermal skin cells in the field is likely to be considerably lower than the authors have considered. The epidermis is protected by the *stratum cornea*.

Human *in vitro* dermal absorption studies for a range glyphosate formulated products are presented in the chapter on dermal absorption, showing a very low dermal absorption of glyphosate; nearly all of the glyphosate is washed off the skin surface after 24 hour exposures (88% to >99 % before *stratum cornea* removal). Therefore, the studies of Heu et al., while

representative of glyphosate spray concentrations, are approximately two or more orders of magnitude higher of those which may result for 8-24 hour dermal exposures.

Author(s)	Year	Study title
Axelrad, J.C. Howard, C.V. McLean, W.G.	2003	The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon Toxicology, Volume: 185, Pages: 67-78 ASB2012-11553

Abstract*

Speculation about potential neurotoxicity due to chronic exposure to low doses of organophosphate (OP) pesticides is not yet supported by experimental evidence. The objective of this work was to use a cell culture model of chronic OP exposure to determine if such exposure can alter the sensitivity of nerve cells to subsequent acute exposure to OPs or other compounds. NB2a neuroblastoma cells were grown in the presence of 25 µM diazinon for 8 weeks. The OP was then withdrawn and the cells were induced to differentiate in the presence of various other pesticides or herbicides, including OPs and OP-containing formulations. The resulting outgrowth of neurite-like structures was measured by light microscopy and quantitative image analysis and the IC₅₀ for each OP or formulation was calculated. The IC₅₀ values in diazinon-pre-exposed cells were compared with the equivalent values in cells not pre-exposed to diazinon. The IC₅₀ for inhibition of neurite outgrowth by acute application of diazinon, pyrethrum, glyphosate or a commercial formulation of glyphosate was decreased by between 20 and 90 % after pre-treatment with diazinon. In contrast, the IC₅₀ for pirimiphos methyl was unaffected and those for phosmet or chlorpyrifos were increased by between 1.5- and 3-fold. Treatment of cells with chlorpyrifos or with a second glyphosate-containing formulation led to the formation of abnormal neurite-like structures in diazinon-pre-exposed cells. The data support the view that chronic exposure to an OP may reduce the threshold for toxicity of some, but by no means all, environmental agents.

* Quoted from article

Klimisch Evaluation

Reliability of study:

Not reliable

Comment:

Incorrect characterisation of glyphosate as an organophosphate pesticide. Inappropriate test system for formulations containing surfactant; cytotoxic membrane disruption potential of surfactant are well known for in vitro test systems. Exposure route not relevant for human risk assessment. Rationale for chosen test substance concentration not given.

Relevance of study:

Not relevant (*in vitro* data, do not reflect real *in vivo* exposure situations. Pre-exposure to diazinon is not relevant for this submission).

Klimisch code:

3

Author(s)	Year	Study title
Benedetti, A. L.	2004	The effects of sub-chronic exposure of Wistar rats to the

Vituri, C.D. Trentin, A.G. Domingues, M.A.C. Alvarez-Silva, M.	herbicide Glyphosate-Biocarb® Toxicology Letters, Volume: 153, Pages: 227-232 ASB2012-11562
---	---

Abstract*

The object of this study was to analyze the hepatic effects of the herbicide Glyphosate-Biocarbo (as commercialized in Brazil) in Wistar rats. Animals were treated orally with water or 4.87, 48.7, or 487 mg/kg of glyphosate each 2 days, during 75 days. Sub-chronic treatment of animals starting from the lowest dose of glyphosate induced the leakage of hepatic intracellular enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggesting irreversible damage in hepatocytes. We observed the increase of Kupffer cells in hepatic sinusoid of glyphosate-treated animals. This was followed by large deposition of reticulin fibers, composed mainly of collagen type III. We may conclude that Glyphosate-Biocarbo may induce hepatic histological changes as well as AST and ALT leaking from liver to serum in experimental models.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comments:

Study report meets basic scientific principles. Study design and documentation is insufficient for assessment.

Relevance of study:

Not relevant because study design not sufficient for assessment of toxicity of the active substance Glyphosate. Toxicity is attributable to high oral dosing of surfactant component. There are several reporting deficiencies.

Klimisch code:

3

Author(s)	Year	Study title
Mesnager, R. Clair, E. Gress, S. Then, C. Szekacs, A. Seralini, G.E.	2012	Cytotoxicity on human cells of Cry1Ab and Cry 1Ac <i>Bt</i> insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology doi: 10.1002/jat.2712. [Epub ahead of print] ASB2012-11900

Abstract*

The study of combined effects of pesticides represents a challenge for toxicology. In the case of the new growing generation of genetically modified (GM) plants with stacked traits, glyphosate-based herbicides (like Roundup) residues are present in the Roundup-tolerant edible plants (especially corns) and mixed with modified *Bt* insecticidal toxins that are produced by the GM plants themselves. The potential side effects of these combined pesticides on human cells are investigated in this work. Here we have tested for the very first time Cry1Ab and Cry1Ac *Bt* toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293, as well as their combined actions with Roundup, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release

by membrane alterations and caspase 3/7 inductions. Cry1Ab caused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. The Roundup tested alone from 1 to 20 000ppm is necrotic and apoptotic from 50ppm, far below agricultural dilutions (50% lethal concentration 57.5ppm). The only measured significant combined effect was that Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by Roundup; this could delay the activation of apoptosis. There was the same tendency for the other markers. In these results, we argue that modified *Bt* toxins are not inert on non-target human cells, and that they can present combined side-effects with other residues of pesticides specific to GM plants.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline, non-GLP <i>in vitro</i> tests meeting scientific principles. Deficiencies: No positive controls were specified, test conditions not described (referenced to a description elsewhere). Exceedingly high doses and an inappropriate test system for formulations containing surfactant; cytotoxic membrane disruption potential of surfactants are well known for <i>in vitro</i> test systems.
Relevance of study:	Relevant with restrictions (Due to reliability. The assessed combinatory effects are of limited relevance)
Klimisch code:	3

Additional comments:

Direct exposure to cells in culture bypasses normal processes limiting absorption and cellular exposure and avoids normal metabolism, excretion, serum protein binding, and other factors that would protect cells in the intact organism.

Anadon et al. (2009, ASB2012-11542) dosed rats with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved peak modeled plasma concentrations of glyphosate of approximately 5 ug/mL (5mg/L or 5 ppm). Assuming linear kinetics, the maximum allowable US daily intake (2 mg/kg/day) would give an approximated blood concentration of 0.025 ppm (25 ppb). McQueen et al. (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4 % of the acceptable daily intake.

The “Roundup” LC₅₀ concentration used (57.5 ppm) is more than 2000-fold higher than the anticipated concentration (based on Anadon et al., 2009, ASB2012-11542) following maximum allowable intake.

The co-application of Cry protein with the glyphosate-surfactant reduces the apparent degree of cellular injury (as measured by induction of caspase levels). This occurs even at concentrations of Cry1Ab which the authors report to cause cellular injury and membrane disruption. This is worth noting for several reasons:

First, it brings into question the toxicity observations with Cry1Ab, as the argument that membrane disruption and impaired mitochondrial function should be protective seems to be highly untenable, especially in view of the studies (Levine et al, 2007, ASB2009-9030) demonstrating the mitochondrial membrane activity of surfactants.

Second, it should take off the table any implications of a “synergistic effect” of Cry proteins and glyphosate-surfactant herbicides. (The direction is, if anything, antagonistic, but the entire system is fundamentally irrelevant.)

Third, this probably is demonstrating the artificiality of the system itself. As noted above, this is a protein-free medium. Protein protects cells in culture by multiple mechanisms- binding to toxic materials, binding to potential receptor sites, or other non-specific surface-stabilisation effects. It appears from Mesnage’s own data that simple addition of protein to their system, even at low concentrations (and even if that protein is a Cry protein) protects from toxicity.

Author(s)	Year	Study title
Clair E., Linn, L., Travert, C., Amiel, C., Seralini, G.E	2012	Effects of Roundup® and Glyphosate on Three Food Microorganisms: <i>Geotrichum candidum</i> , <i>Lactococcus lactis</i> subsp. <i>cremoris</i> and <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> Current Microbiology, Volume: 64 Number: 5, Pages: 486-491 ASB2012-11592

Abstract*

Use of many pesticide products poses the problem of their effects on environment and health. Amongst them, the effects of glyphosate with its adjuvants and its by-products are regularly discussed. The aim of the present study was to shed light on the real impact on biodiversity and ecosystems of Roundup®, a major herbicide used worldwide, and the glyphosate it contains, by the study of their effects on growth and viability of microbial models, namely, on three food microorganisms (*Geotrichum candidum*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) widely used as starters in traditional and industrial dairy technologies. The presented results evidence that Roundup® has an inhibitory effect on microbial growth and a microbicide effect at lower concentrations than those recommended in agriculture. Interestingly, glyphosate at these levels has no significant effect on the three studied microorganisms. Our work is consistent with previous studies which demonstrated that the toxic effect of glyphosate was amplified by its formulation adjuvants on different human cells and other eukaryotic models. Moreover, these results should be considered in the understanding of the loss of microbiobiodiversity and microbial concentration observed in raw milk for many years.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Non-validated, non-guideline test with methodological and reporting deficiencies (e.g. dose concentrations in media not specified, no positive controls or controls that show the validity of the test system /and concentration range tested). Inappropriate test system for formulations containing surfactant; cytotoxic membrane disruption potential of surfactants are well known for in vitro test systems.

Relevance of study:

Not relevant (Due to reliability)

Klimisch code:

3

Additional Comments

Glyphosate at 1 % had no effect on lactobacilli but did impair *Geotrichum*, which is unsurprising as glyphosate at herbicidal concentrations will impact metabolism of many fungi, which (like plants) use the shikimate pathway for aromatic amino acid production.

Surfactants are known to be bacteriostatic, with (for example) quaternary ammonium compounds typically being active in the 30-150 ppm range.

Clair et al. demonstrate that surfactants are bacteriostatic for 3 microorganisms at concentration ranges well within the range of concentrations generally found to be useful for sanitation purposes. However, surfactant solutions are routinely used to sanitize food processing equipment at concentrations at or above those tested by Clair et al. (2012, ASB2012-11592).

B.6.9 Medical data and information (Annex IIA 5.9)

B.6.9.1 Report on medical surveillance on manufacturing plant personnel

Industrial hygiene air monitoring data for glyphosate with workers at the Monsanto Luling, Louisiana manufacturing facility are available for the years 1981-1998 and have been submitted as part of the GTF dossier (no particular reference available). No such data are available from a Monsanto European manufacturing facility. Based on the measured low exposures to glyphosate in the manufacturing setting (well below the ADI) and low toxicological concern, glyphosate specific medical monitoring was not considered necessary by Monsanto. The following data are air concentration measurements which are conservatively applied as 100 % bioavailable to calculations of mean and maximum daily exposures.

Table B.6.9-1: Particulate exposures from glyphosate technical acid operations involving wetcake, e.g., supersack or container filling operations. Values are time weighted averages

Glyphosate Technical Dust (mg/m ³)					Mean Daily Exposure* (mg/kg/day)	Maximum Daily Exposure* (mg/kg/day)
Sample Type	# Samples	Range	Mean	SD		
All	179	0.0003-0.2594	0.00647	0.0218	0.00108	0.04323
Personal	176	0.0003-0.2549	0.00655	0.022	0.00109	0.04248
Area	3	0.0008-0.024	0.00153	0.0008	0.00026	0.00400
Operator	158	0.0008-0.2594	0.00727	0.0235	0.00121	0.00393
Maintenance	16	0.0005-0.0053	0.00206	0.0014	0.00034	0.00088
Lab	2	0.0003-0.0004	0.00035	N/A	0.00006	0.00007

* based on breathing 10 m³ air/shift and 60 kg worker

Table B.6.9-2: Glyphosate isopropylamine salt liquid formulation bottling, drumming and tote filling operations. Values are time weighted averages

Glyphosate IPA Salt- Liquid Formulations (mg/m ³)					Mean Daily Exposure** (mg/kg/day)	Maximum Daily Exposure** (mg/kg/day)
Sample Type	# Samples	Range	Mean	SD		
All	72	0.0001-0.47	0.085	0.105	0.01050	0.05804
Personal	58	0.0001-0.47	0.0251	0.106	0.00310	0.05804
Area	14	0.004-0.28	0.0932	0.105	0.01151	0.03458
Operator	54	0.0001-0.47	0.0966	0.11	0.01193	0.05804
Maintenance	4	0.0041-0.0088	0.00792	0.00187	0.00098	0.00099

** based on breathing 10 m³ air/shift and 60 kg worker and divided by 1.3496 to convert IPA salt to technical acid

Improvements in manufacturing facility containment and ventilation systems over recent years further reduce the likelihood of operator exposures within glyphosate manufacturing facilities.

B.6.9.2 Reports on clinical cases and poisoning incidents

Glyphosate is worldwide used extensively as herbicide. According to this extensive use a large number of poisoning incidents happened and was published. An extensive review of clinical cases was submitted by [REDACTED] 2004, ASB2012-11576. Another review of cases was published by [REDACTED] 2008, ASB2012-11879.

[REDACTED] (2009, ASB2013-11831) briefly mentioned a total of 60 reports by physicians from Germany on cases of poisoning with glyphosate herbicides since 1990. Without further specification, in the vast majority of 52 cases only slight health impairment was reported. In four cases, health disturbances were considered “moderate” whereas the only one actually life-threatening case was the result of ingestion of 200 mL of a herbicide containing glyphosate and a [REDACTED] surfactant with suicidal intent. In the three remaining cases, no symptoms were reported or their severity could not be evaluated.

More than 650 cases of intoxication/irritation ascribed to ingestion of/contact to glyphosate-based herbicides are mentioned in an overview on poisoning incidents from Brazil that was just recently kindly provided to the RMS by the Brazilian National Health Surveillance Agency (Paumgarten/ANVISA, 2012, ASB2013-13413). This data was collected by up to 10 Brazilian poison centers between 2010 and 2012. It is not clear if it is representative for the whole huge country in which the agricultural conditions in general and also those of pesticide use are extremely different. In addition, there is even a higher number of poison information centers in Brazil that have not provided data and, thus, the number of incidents might underestimate the real incidence. On the first glance, the exposure routes, ingested amounts, circumstances (accident, suicidal attempt?), clinical signs and medical treatment are similar to what is known from Germany and from the literature. The much higher total number of cases (as compared to Germany) seems to reflect the applied amount of glyphosate and its formulations that is by orders of magnitude higher in Brazil. Unfortunately, severity of symptoms is not graded and very often the final outcome was not reported. Another problem is that the product to which exposure was claimed is not specified. Frequently, “Roundup” is mentioned but different formulations are marketed under this name. In many other cases, the incident is ascribed to “glyphosate”. However, this is not credible because there is no simple access to the active ingredient and a certain product must have been applied. Furthermore, it is not clear whether a causal relationship had been actually confirmed. Thus, this potentially most interesting data require further thorough analysis before it may be used for evaluation of health risks.

B.6.9.3 Observations on exposure of the general population and epidemiological studies

A number of human studies on genotoxicity have been published since 2000 in which exposures of the studied populations to glyphosate-based formulations were postulated. These publications are presented and discussed in chapter B.6.4 (Genotoxicity), in the “Published data” section under B.6.4.8.7 (Human and environmental studies).

Likewise, several epidemiology studies on a possible relationship of exposure to glyphosate (and further pesticides) and cancer have been published since 2000 that are presented and discussed in chapter B.6.5 under “Published data” (B.6.5.3 Published data).

With regard to reproductive outcome in humans, a number of epidemiological studies in which glyphosate exposure was considered are presented and discussed in chapter B.6.6 (Reproductive toxicity).

Systemic exposure of humans following field application of glyphosate or presumed dietary intake may be roughly calculated on the basis of urinary concentrations. Two studies on concentrations of glyphosate in human urine samples (██████████ 2004, ASB2012-11528) and ██████████ (2013, ASB2013-8037) are available and reported in detail in chapter B.6.1 under B.6.1.3 (Published information). For evaluation of this data and comparison of the resulting assumptions for systemic doses that were received by the participants with the proposed reference values, see Vol. 1 (2.6.2).

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

The summary in this section is based on well over 30 years of experience with numerous formulations of glyphosate in a wide range of situations. The extensive use of glyphosate has encouraged clinical assessment of various interventions and has resulted in reporting of alleged associations of symptoms with exposures to glyphosate products. The clinical toxicology of glyphosate and of glyphosate-surfactant formulations have been the subject of an extensive review (██████████ 2004, ASB2012-11576), and a review of cases with assessment of clinical prognostic factors was more recently published (██████████, 2008, ASB2012-11879).

Animals do not have the shikimic acid pathway; and no direct target-mediated mode of action in mammalian systems has been clearly identified to date (██████████ 2004, ASB2012-11576). Glyphosate does not inhibit the cholinesterases, and has no cholinergic effect. While incidental exposure in glyphosate-surfactant herbicide mixtures is common, review of available case reports (AAPCC 2003-2011) indicates that the vast majority of reported non-suicidal exposures involve skin and/or eye irritation or irritation of the respiratory tract by inhalation of spray mist, and that systemic symptoms are rare following non-suicidal exposures to glyphosate products. Based upon human experience and animal data, even those systemic symptoms reported following incidental exposure appear unlikely to be causally related to exposure (██████████ 2002, ASB2012-11831).

The following clinical effects are divided into those expected following minor and significant exposures for each category based upon expected severity of systemic symptoms. The factors which determine if the exposure was minor or significant include:

- Route of exposure.

Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures for purposes of the symptom descriptions below. Ingestions more than 50 mL (one mouthful if amount unknown) of a product with >10 % glyphosate concentration may be significant.

- Concentration of the product.

Glyphosate concentrations of less than 10 % rarely if ever produce significant toxicity. Most serious illness has historically resulted from ingestion of the 41 % (glyphosate IPA) concentrate. In the absence of extensive clinical experience for the 11-40 % concentration range, any ingestion of greater than 50 mL of a glyphosate preparation having a greater than 10% concentration of glyphosate salts should be considered potentially significant for purposes of the symptom descriptions below.

- Intent of the exposure.

Accidental ingestion rarely involves large quantities of concentrated formulations. Intentional ingestion cases may not present with a reliable history and may require observation if the amount ingested cannot be reliably determined.

Route and organ system specific symptoms of exposure:

Dermal

Minor exposures:

Contact with skin may produce a dermatitis similar to that of detergents (2004, ASB2012-11576)

It is expected that the severity of injury following skin exposure will be significantly decreased with a less concentrated product and with a reduced duration of contact.

Phototoxic reactions (sunlight or ultraviolet (UV) light induced skin reactions) have been reported. These symptoms are believed to be due to an antimicrobial additive (benzisothiazolone) which is present in selected residential use (i.e. non- agricultural) products containing 10 % glyphosate or less (2004, ASB2012-11576).

Significant absorption through the skin does not occur (see also B.6.12).

Significant exposures:

Skin exposures are not expected to cause systemic effects or serious cutaneous effects when the results of animals studies and the low dermal absorption are taken into account. There are no reports that would suggest the contrary. Symptoms as noted in the minor exposure may occur.

Ocular

Minor exposures:

A review of ocular exposures to US glyphosate-surfactant formulations (1513 exposures over a 5-year period), showed no permanent eye injury (1999, TOX2002-699).

Human eye exposures have generally resulted in temporary conjunctival irritation, clearing after irrigation or in 1-2 days, and permanent eye damage is said to be “most unlikely” (2004, ASB2012-11576).

It is expected that the severity of injury following eye exposure will be significantly decreased with a less concentrated product or with a reduced contact time.

Significant exposures:

Eye exposures are not expected to cause systemic effects or serious ocular injury (1999; TOX2002-699, 2004, ASB2012-11576).

Systemic exposure – ingestion or inhalation

- Neurologic

Minor exposures:

There is no clinical or experimental evidence that glyphosate or glyphosate-surfactant formulations cause neurological symptoms or injury after exposure by any route.

Significant exposures:

There have been no reports of primary convulsions after ingestion.

One author reports most patients present with a clear sensorium unless another substance, such as alcohol, has been co-ingested or severe hypoxemia has occurred (1989,

TOX9552426); however "moderate disorders of consciousness" have been reported within 48 hours of suicidal ingestions of the concentrate (██████████ 1987, Z35531; ██████████ 1988, Z35532). This has occurred in patients with significant systemic illness and is not believed to be the result of reduced organ perfusion (██████████ 2004, ASB2012-11576) or perhaps other factors such as metabolic disturbance but the possibility of a direct toxicological effect cannot be excluded (██████████ 2004, ASB2012-11576). There are two isolated case report of Parkinson's disease developing in individuals with a history of glyphosate product exposure (██████████ 2001, ASB2012-11557; ██████████ 2011, ASB2012-12047). These publications are reported in detail with Klimisch rating in chapter B.6.7 (Neurotoxicity) under B.6.7.2 (Published data), because they are discussed there in context with other studies on neurotoxicity and on Parkinson's disease.

- Gastrointestinal:

Minor exposures:

Minor exposures are likely to be asymptomatic, but the patient may experience an unpleasant taste, tingling, mild self-limited nausea and vomiting.

Self-limited diarrhoea may also occur which is thought to be due to the surfactant.

Significant exposures:

A burning sensation in the mouth and throat, salivation, oral erythema, sore throat, dysphonia, dysphagia, epigastric pain, nausea, spontaneous vomiting, abdominal pain and diarrhoea are common and may last up to a week.

Serum amylase may be elevated; isoenzyme analysis done in a few cases identified a salivary gland origin (██████████ 1989, TOX9552426).

In severe cases with large ingested doses, hematemesis, GI bleeding, melena and hematochezia may occur. Paralytic ileus has been reported as a rare event.

Endoscopy has noted erosions of the pharynx and larynx, esophagitis and gastritis with mucosal oedema, erosions and haemorrhage. However, transmural injury and perforation have not been noted (██████████ 1999, ASB2012-11510).

In fatal cases, autopsy notes mucosal or transmural oedema and necrosis throughout the small bowel with erosion and haemorrhage; in the large bowel, mucosal oedema and focal haemorrhage was noted (██████████, 1989, TOX9552426).

Clinical, autopsy and experimental evidence (██████████, 1987, TOX9552430) indicate a potential for gastrointestinal damage from glyphosate components of glyphosate formulations, but the frequency of severe injury appears to be low.

- Cardiovascular:

Minor exposures:

Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Cardiovascular effects are not expected to result from such minor exposures and no reports are available.

Significant exposures:

Hypotension is common after ingestions of a mouthful or more of the concentrated product (not the diluted forms) and usually responds to IV fluids and pressor amines. Shock as manifested by oliguria, anuria and hypotension which was unresponsive to fluids and pressors, ultimately resulting in death, has been reported. (██████████ 1989, TOX9552426, ██████████ 2004, ASB2012-11576). Transient hypertension may be noted.

- Upper respiratory:

Minor exposures:

Dermal, eye and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Significant upper respiratory effects are not expected from minor exposures, but minor irritation or discomfort may occur (██████████ 2004, ASB2012-11576).

Significant exposures:

Significant systemic exposures are not anticipated to occur via the inhalational route. However, if occurring, they would most probably also affect the lower respiratory tract (see below).

- Lower respiratory:

Minor exposures:

Because of the non-volatile nature of glyphosate and the surfactant exposures to vapour is not possible. The spray equipment that is commonly used will produce particles that are non-respirable.

Significant exposures:

Tachypnea, dyspnea, cough and bronchospasm including cyanosis have been seen in severe ingestions (more than a mouthful of concentrated product). These effects are indicative of systemic toxicity.

Aspiration pneumonia, pulmonary oedema and respiratory failure have been seen although the exact role of aspiration has not been fully investigated.

An isolated case report suggests the development of acute pneumonitis in a worker following his performing maintenance on non-operating spray equipment used to apply a glyphosate-surfactant formulation (██████████ 1998, ASB2012-11513). However, actual exposure and its extent could not be really substantiated in this case. Accordingly, the occurrence of pneumonitis in this individual is more likely to be coincidental by nature although a (different) occupational origin seems plausible (██████████ 1999, ASB2012-11511).

There is also a case report from Germany in which a glyphosate-surfactant product (██████████ or “POEA” based) was applied by knapsack sprayer in a 0.5ha forestry application at the registered application rate at 25° C for approximately 3 hours without respiratory protection (██████████ 2009, ASB2013-11831). About 7 hours after application he developed chest pain with rapidly increasing severe respiratory distress and fever up to approximately 38° C. On hospital admission, radiographic changes of lungs could be demonstrated. To further assess possible causes, bronchoscopy and closed lung biopsy was performed. Histopathology revealed “toxic inflammation of the lungs” that was significantly different from bacterial infection. After 7-days of drug treatment, changes in lung reversed but six months after the incident, the patient still experienced moderate respiratory complaints on exertion. In the X-ray examination, there were still detectable lung changes although some improvement had been noted. In addition, in the same reference, 20 cases of inhalative exposure among a total of 60 reports on confirmed or presumed poisoning incidents with glyphosate herbicides from Germany (since 1990) were mentioned with breathing difficulties occurring in 50 % of the affected people. No more details on clinical courses or outcomes were given but it was emphasised by the authors as “striking” that the involved products nearly always contained ██████████

- Renal:

Minor exposures:

Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Renal effects are not expected to result from such minor exposures and no reports are available.

Significant exposures:

Hypotension and hypovolemic shock may result in oliguria and anuria, following severe ingestions (██████████ 2004, ASB2012-11576). Abrupt rises in BUN and serum creatinine may be seen.

- Metabolic:

Minor exposures:

Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Metabolic effects are not expected following minor exposures and no reports are available.

Significant exposures:

Mild fever may be noted even in the absence of infection (██████████ 2004, ASB2012-11576)

Metabolic acidosis is often seen in a severely poisoned patient (██████████ 2004, ASB2012-11576) and this acidosis may fail to respond to bicarbonate therapy. Although the exact nature was not elucidated, a lactic acidosis was suspected.

- Hematologic:

Minor exposures:

Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Haematological effects are not expected from minor exposures and no reports are available.

Significant exposures:

Leukocytosis without evidence of bacterial infection has been noted in peripheral blood after ingestion of the concentrate (██████████ 2004, ASB2012-11576).

Hemoconcentration has been seen as a result of intravascular volume depletion and might indicate severe capillary fluid leakage (██████████ 1989, TOX9552426).

No primary toxic effects on bone marrow or formed elements have been reported to date.

- Hepatic:

Minor exposures:

Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Hepatic effects are not expected from such minor exposures and no reports are available.

Significant exposures:

No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin are reported (██████████. 1989, TOX9552426; ██████████ 2004, ASB2012-11576).

- Clinical chemistry (electrolytes):

Minor exposures:

Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance. However, such signs are not expected following a minor exposure.

Significant exposures:

Electrolytes (Na, K, Cl and Ca) in the absence of renal failure generally remain normal. Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance.

POTASSIUM SALTS: While potentially toxic ingestions of all glyphosate products may result in fluid and electrolyte disturbances, particular attention to potassium may be important following ingestion of the potassium salt products. Close monitoring of serum potassium levels and/or electro-cardiographic monitoring (for peaked T-waves or rhythm disturbances) is recommended following significant ingestion of potassium salt products, particularly for high risk individuals. Individuals with the following may be at elevated risk following acute potassium exposure: known hyperkalemia, renal failure / renal dysfunction, use of potassium sparing diuretics, hypoaldosteronism, co-ingestion of other K⁺ containing materials, underlying heart disease, use of digoxin, digitoxin, oabain, or exposure to other cardiac glycosides. The quantity of potassium ingested from a glyphosate potassium salt product can be estimated from the weight percent of glyphosate potassium as:

Percent K⁺ salt x 5.3 = mEq potassium per 100 cc of product

Several case reports indicate that after ingestions of large amounts of glyphosate-potassium salt concentrate solutions, clinically significant hyperkalemia may occur. [REDACTED] (2001, ASB2012-11556) reported an intoxication in a 65 year old female who ingested a glyphosate-potassium salt (350 mL Roundup Maxload missing from container, in addition to 250 mL of another glyphosate formulation which was not a potassium salt but amount actually ingested unclear) in a suicidal attempt. On admission, serum potassium level was 9.3mEq/L (typical normal value < 5) with electrocardiographic changes consistent with hyperkalemia. The patient did have a concomitant acidosis (pH 7.272) which may account for some portion of the elevation in potassium (acidosis displaces intracellular potassium). The patient responded to medical management and survived.

[REDACTED] (2012, ASB2012-11863) reported the case of a 69 year old female who ingested approximately 500 mL of the same product. On arrival in the hospital, the patient had hyperkalemia (10.7 mEq/L), pulseless ventricular tachycardia, and a severe metabolic acidosis (pH 7.005, will elevate potassium.) The patient required aggressive cardiopulmonary resuscitation and hemodialysis but did recover.

According to the GTF dossier (no particular reference given), Monsanto is aware of one additional (unpublished) case of a similar ingestion with dramatically elevated potassium level in which the patient was moribund when medical care was instituted. The patient could not be resuscitated. Because serum potassium levels rise rapidly following death (due to redistribution of intracellular potassium), it is not possible to know how much of the observed hyperkalemia was the result of the ingestion versus profound acidosis and *post mortem* redistribution (which is partially due to acidosis).

It should be noted that the issue of hyperkalemia is limited to cases involving the suicidal ingestion of glyphosate-potassium concentrates. Potassium is a normal component of the human diet, and potassium intake attributable to occupational glyphosate-surfactant herbicide exposure will be negligible compared to typical dietary intake. While the concentrate

formulations may contain up to approximately 250 mEq of potassium per 100 mL, product diluted for use (1 % glyphosate concentration) will contain about 6 mEq potassium per 100 mL. By way of reference, a medium size banana contains about 10 mEq (425 mg) of potassium.

Finally, it should be noted that the apparently very large (>150 mL) ingestions of glyphosate-surfactant concentrates observed in these cases are well within the range isopropylamine salt products reported to produce fatalities, and that elevations in potassium concentrations are reported (probably due to acidosis) following ingestions of glyphosate IPA salt products. While the cases do suggest that potassium salt products likely contribute to the risk of hyperkalemia, it is not clear at this time if the use of potassium salts will increase the overall clinical severity and/or mortality associated with glyphosate concentrate product ingestions.

Specific diagnostic testing and prognostic considerations

Serum or other body fluid measurements of glyphosate will be generally not available in a time frame that would be useful for acute clinical diagnosis. As the management of symptoms associated with glyphosate-surfactant product ingestion is symptom-driven in any event, the lack of rapidly available knowledge on concentrations of glyphosate will generally not impair clinical care. Levels may be more helpful in addressing forensic issues.

Attention should be paid to electrolyte concentrations in individuals with significant ingestion exposures, particularly to glyphosate-potassium concentrate solutions.

Respiratory distress requiring intubation, pulmonary oedema, shock (systolic BP <90 mmHg), altered consciousness, abnormal chest X-ray, ingestion of over 200 cc concentrate (41 %), or renal failure necessitating dialysis have been associated with a higher risk of poor clinical outcomes including mortality (█ 2008, ASB2012-11879). A prognostic index based upon these factors was developed but its use is not expected to contribute significantly to improved medical care. As symptom onset may be delayed, early use of such prognostic indicators may in fact result in under-estimation of clinical severity of a case.

B.6.9.5 First aid measures

The following, quite general measures have been proposed by notifiers but were not evaluated by RMS toxicologists because this is beyond the scope of this RAR :

Skin exposure:

Remove all contaminated clothing and flood the skin surface with water.

Wash the exposed skin twice with soap and water.

A close examination of the skin may be required if pain or irritation exist after decontamination.

All clothing that are contaminated should be laundered before they are worn again

Eye exposure:

Remove contact lens from the affected eye(s) if appropriate.

Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.

A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably with an eye specialist.

Ingestion exposure:

Dilute preparations (Glyphosate <10 %): An ingestion of a dilute preparation of glyphosate (<10 %) probably does not require treatment other than dilution with milk or water and symptomatic care. Further gastrointestinal decontamination is not needed, even if spontaneous emesis has not occurred.

Concentrated (> 10 %) preparations: Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 mL/kg in a child or 250 mL in an adult.

Inhalation exposure:

No pulmonary treatment is necessary for occasional, accidental breathing of mist.

Severe, acute pulmonary injury has not been reported following inhalation exposure. Individuals with respiratory distress from any cause should be relocated (if medically stable) to fresh air and receive supplemental oxygen if available.

In the event of respiratory failure or lack of respiration, administer artificial respiration (or if pulse not detectable, cardiopulmonary resuscitation).

B.6.9.6 Therapeutic regimes

The following therapeutic regimes have been proposed by notifiers but were not evaluated by RMS toxicologists because this is beyond the scope of this RAR :

“The registrants believe that the following represent general best practices for medical management of serious ingestions of glyphosate-surfactant products.

Establish respiration and assure adequacy of ventilation.

Eye exposure:

Remove contact lens from the affected eye(s) if appropriate.

Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.

A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably by an eye specialist.

Ingestion exposure:

Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 mL/kg in a child or 250 mL in an adult.

patient disposition:

Concentrated preparations (Glyphosate 41 % or greater):

Any person ingesting greater than a large mouthful (50 mL in an adult, 0.5 mL/kg in a child) of a 41 % or greater glyphosate concentrate product should be admitted to a hospital and observed for 24 hours.

Any adult ingesting greater than 100 mL of a 41 % or greater glyphosate concentrate product (>1.4 mL/kg in a child) should be admitted to the intensive care unit.

Any suicide attempt by person ingesting a concentrated product should be evaluated for psychological status and should be admitted if necessary for observation with suicide precautions.

Concentrated preparations (Glyphosate 10 %-40 %):

An ingestion of concentrated glyphosate (10 % - 40 %) will usually result in spontaneous emesis. There is limited experience with glyphosate formulations in this concentration range. In view of this limited information, the registrants currently recommend managing these ingestions in a manner similar to the management of the 41 % concentrate.

Prevention of absorption (*This lists various methods for "Prevention of Absorption". These should NOT be construed as being in order of preference. Consult with Poison Center or medical personnel to determine the need for and preferred method for decontamination. In many instances, no intervention is required.*)

Gastric aspiration: If no significant spontaneous vomiting has occurred gastric aspiration may be considered. If performed soon after ingestion, gastric emptying by aspirating liquid gastric content with a lavage or standard NG tube may possibly remove some of the ingested glyphosate. The intent is to remove unabsorbed liquid by aspiration not to use lavage fluid. As absorption of liquids is likely to be relatively rapid, gastric aspiration after 1 to 2 hours is unlikely to be effective.

Emesis: Emesis is controversial at this time. Glyphosate/surfactant products are irritants. The registrants do not recommend the routine use of syrup of ipecac for glyphosate / surfactant ingestions because of the risk of exacerbating the irritant effects on the GI tract.

Activated charcoal: There are no data to support or refute the use of activated charcoal in glyphosate/surfactant product ingestions. Low molecular weight, amphoteric compounds and detergents do not always bind well to activated charcoal. In the event of a mixed ingestion, activated charcoal may be advisable.

Assessment of gastro-intestinal injury

Injury to the upper gastrointestinal tract may occur following ingestion of glyphosate concentrates. A study of upper gastrointestinal endoscopy following glyphosate-surfactant ingestions suggested that Zarger grade 2 lesions (erosions) were associated with longer hospital stay and with a higher incidence of serious complications (Chang 1999, ASB2012-11510). However, no major esophageal or gastrointestinal injury was observed, and strictures have not been reported following uncomplicated glyphosate-surfactant ingestion.

Because no serious gastrointestinal injury is reported, and because the need for hospitalisation and/or treatment of complications can be determined without endoscopic evaluation, the registrants recommend that endoscopy be reserved for patients with co-ingestions suggesting a need for endoscopy or for patients with signs and symptoms suggestive of more serious injury (serious oral burns, inability to handle secretions, clinical obstruction) regardless of clinical history.

Monitor blood pressure:

Monitor the patient closely for signs of hemodynamic instability. The insertion of a Swan-Ganz catheter may be warranted.

Hypotension:

If the patient is hypotensive, administer IV fluid boluses and place in Trendelenburg position. If the patient is unresponsive to these measures, administer a vasopressor (dopamine, epinephrine, norepinephrine, phenylephrine, isoproterenol, etc.) if needed.

Monitor blood gases and obtain chest radiograph:

Consider the use of repeat blood gases and a peripheral pulse oximeter to monitor hypoxemia. Observe closely for sign of acidosis.

Pulmonary oedema:

Closely monitor arterial blood gases. If PO₂ cannot be maintained above 50 mm Hg with inspiration of 60 % oxygen by face mask or mechanical ventilation, then positive end expiratory pressure (PEEP) or continuous positive airway pressure (CPAP) may be needed. Avoid a positive fluid balance by careful administration of crystalloid solutions. Monitor fluid status through a central venous line or Swan Ganz catheter as needed.

Acidosis:

Correction of acidosis should be guided by blood gases, electrolytes and clinical judgment. Attention should be directed to volume status and correction of poor perfusion in mild cases. Sodium bicarbonate may be used to correct the acidosis in severe cases.

Hyperkalemia (from ingestion of Potassium salt formulations):

For moderate hyperkalemia (K⁺ of 6.0-7.0 mEq/L), administer sodium polystyrene sulfonate with sorbitol. For more severe hyperkalemia (K⁺ > 7 mEq/L) or serious complications of hyperkalemia, correct metabolic or respiratory acidosis if present to allow potassium to enter the intracellular space. Additional management may include a glucose/insulin drip, intravenous sodium bicarbonate or calcium, and dialysis to remove excess potassium.

Monitor renal function closely:

Assure adequate urine output. Catheterise severely ill patients. Hemodialysis may be needed in the event of renal failure or electrolyte disturbances.

Enhanced elimination:

Forced diuresis: Glyphosate is excreted very well by the kidneys. Adequate urine flow will ensure the rapid elimination of glyphosate. Although elimination may perhaps be enhanced by forced diuresis, there is no clinical evidence that this is necessary, and fluid overload may precipitate pulmonary oedema.

Hemodialysis: Hemodialysis may be useful to correct fluid, electrolyte and metabolic disturbances in the patient with renal failure. The institution of hemodialysis solely to enhance the removal of glyphosate or other product components is not of proven benefit. Nevertheless, it is reasonable to consider the initiation of hemodialysis in the significantly ill patient who fails to respond to routine supportive management.

Serious exposure via inhalation is not expected:

Inhalation exposures are not expected due to the aerodynamics of droplet size from sprayers and because the product is not volatile. Monitor the patient for signs of respiratory compromise. Create an artificial airway if necessary. Check adequacy of tidal volume. Monitor the patient for respiratory distress; if a cough or dyspnea develop, evaluate the patient for respiratory irritation, bronchitis and/or pneumonia, but these are not expected.

Serious exposure via skin is not expected:

Significant skin exposures are not expected; however, the patient should be treated empirically if a dermal exposure is suspected. Remove all contaminated clothing and flood the skin surface with water. Wash the exposed skin twice with soap and water. A close

examination of the skin may be required if pain or irritation exist after decontamination. All contaminated clothing should be laundered before wearing.

Laboratory:

Monitor electrolytes, especially if the patient is experiencing vomiting and diarrhea.¹⁵ Patients ingesting concentrated products based on the potassium salt of glyphosate may ingest large amounts of potassium (see calculations above). Observe serum potassium and/or electrocardiogram carefully. Patients experiencing pulmonary symptoms or having chest radiograph changes should have arterial blood gas monitoring. A peripheral pulse oximeter and a Swan Ganz catheter may be needed.”

B.6.9.7 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion

Dermal exposure:

Skin irritation following exposure to glyphosate-only or glyphosate-surfactant materials is generally limited to topical irritation which will resolve within 3 days to 1 week following exposure. If exposure is aggravated by occluded conditions or physical abrasion, more severe skin injury with open skin injury may rarely result and may take longer to fully resolve.

Eye exposure:

Irritant symptoms generally resolve within 3-7 days of exposure. Most irritation is minor, but exposure to concentrate or the occurrence of a foreign body or of abrasions (from rubbing the eye) may result in corneal abrasion requiring topical antimicrobial therapy, often given in conjunction with topical corticosteroids and temporary eye patching to provide symptomatic relief. As noted above, a large study of (U.S.) ocular exposures to glyphosate-surfactant products demonstrated no long term eye injury.

Inhalation exposure:

Glyphosate-surfactant products generally do not contain readily volatile ingredients and thus inhalation exposure is limited to inhalation of agricultural droplets, which will deposit primarily in the upper airway. Resulting irritant symptoms will generally resolve within hours to a few days following exposure.

Ingestion:

Following minor or incidental ingestions, or ingestion of fully diluted formulations, gastrointestinal upset with nausea, vomiting, and diarrhoea may occur. Nausea and vomiting usually resolve within a few hours of ingestion. Diarrhoea may last for several days but is generally not severe. Following a major ingestion, the onset of systemic symptoms may be delayed by several hours. Fatalities due to cardiovascular failure are generally delayed by 12 – 36 hours. For serious but non-fatal cases, primary clinical injury generally is manifest within 72 hours but secondary complications such as infection or respiratory distress syndrome may supervene. The majority of serious but surviving cases will be fully recovered within 7-10 days of ingestion. Individuals with complicated hospital courses may require a more extended and highly variable time to recover.

B.6.9.8 Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment

The outcome of eye, dermal, and inhalational exposures, which are not expected to result in serious injury in any event, will not be significantly altered by delays in medical management. Similarly, minor oral exposures are symptomatically managed and unlikely to result in severe gastrointestinal symptoms. Medical management with intravenous fluids may provide some symptomatic relief in the event of dehydration, but recovery is anticipated in any event.

For serious ingestions having major electrolyte disturbances or life threatening alterations of cardiovascular performance, medical intervention may be life saving. Fortunately, as noted above, the onset of serious symptoms following ingestion is generally delayed by at least several hours, allowing for medical transport in all but the most remote or extreme circumstances.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.11)

For extensive discussion on reference values please see Volume 1.

RMS comment (August 2013):

Despite comments by GTF (please refer to commenting table, July 2013) the justification for deriving the ADI and AOEL based on developmental studies in rabbits as given under 2.6.12 in Vol.1 of the DRAR is still considered convincing and was not changed.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.6.11 Acute toxicity including irritancy and skin sensitisation of preparations (Annex IIIA 7.1)

B.6.11.1 Summary

Table B.6.11-1: Information on MON 52276*

Product name and code	MON 52276
Formulation type	Soluble concentrate (SL)
Active substance (incl. content)	Glyphosate, 360 g/L as its isopropylamine salt, 486 g/L; Remark: This formulation does not contain any [REDACTED]
Function	Herbicide
Product already evaluated as the 'representative formulation' during the first Annex I inclusion	Yes

* Information on the detailed composition of MON 52276 can be found in the confidential part of this dossier, Doc. J (Vol.4).

MON 52276, the lead formulation of the Glyphosate Task Force submission, was one of the representative formulations supporting the 2001 Annex I inclusion of glyphosate. This formulation is still registered in Europe and its composition has not changed.

Justified proposals for classification and labelling

In accordance with Directives 67/548/EEC and 1999/45/EC and according to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 the following classification and labelling with regard to toxicological data is proposed for the preparation:

Table B.6.11-2: Justified proposals for classification and labelling

C&L according to Directives 67/548/EEC and 1999/45/EC	
Hazard symbols:	None
Indications of danger:	None
Risk phrases:	None
Safety phrases:	None
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use.
C&L according to Regulation (EC) No 1272/2008	
Hazard classes, categories:	None
Signal word:	None
Hazard statements:	None
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401]
	'15.8 percent of the mixture consist of ingredients of unknown inhalation

	toxicity.'
--	------------

Table B.6.11-3: Summary of risk assessment for operators, workers, bystanders and residents for MON 52276

	Result	PPE / Risk mitigation measures
Operators	Acceptable	German model - Avoid any unnecessary contact with the product. Misuse can lead to health damage. - Keep out of the reach of children. UK POEM - Avoid any unnecessary contact with the product. Misuse can lead to health damage. - Keep out of the reach of children. - Wear protective gloves when handling the undiluted product. - Wear protective gloves when handling/applying the product ready for application. - Wear an impermeable coverall when applying/handling the product ready for application.
Workers	Acceptable	- Treated areas/crops may not be entered until the spray coating has dried.
Bystanders	Acceptable	None
Residents	Acceptable	None

The risk assessment according to the German model has shown that the estimated exposure towards glyphosate in MON 52276 does not exceed the systemic AOEL for operators, workers, bystanders and residents. No specific PPE is necessary for operators or for workers.

The risk assessment according to the UK-POEM has shown that the estimated exposure towards glyphosate in MON 52276 will not exceed the systemic AOEL for operators. Operator exposure will be below the systemic AOEL only, if prescribed PPE is worn.

B.6.11.2 Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for MON 52276

The conclusions of the 2001 EU evaluation of MON 52276 (acute toxicity profile) are summarised in Table B.6.11-4. All data are still relevant to this submission. However a new dermal sensitisation study was subsequently conducted under GLP conditions, following the revised OECD 406 test guideline (modified Buehler; 9 applications). The new dermal sensitisation study confirms both the results of the previously submitted non-GLP study and the 2001 EU evaluation for this end point.

Table B.6.11-4: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for MON 52276

Annex point (2001 EU Monograph Annex Point)	Type of test, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Dir. 67/548/EEC)	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
IIIA 7.1.1 (B.5.11.1.1)	LD ₅₀ oral, rat (OECD 401)	> 5000 mg/kg bw	Yes	None	None	[REDACTED] 1991 TOX9552438
IIIA 7.1.2 (B.5.11.1.2)	LD ₅₀ dermal, rat (OECD 402)	> 5000 mg/kg bw	Yes	None	None	[REDACTED] 1991 TOX9552439
IIIA 7.1.3 (B.5.11.1.3)	LC ₅₀ inhalation, rat	Not submitted, not necessary. Justification presented in Vol. 3, B.6.11.5)				
IIIA 7.1.4 (B.5.11.1.4)	Skin irritation, rabbit (OECD 404)	Non-irritant	Yes	None	None	[REDACTED], 1991 TOX9552440
IIIA 7.1.5 (B.5.11.1.5)	Eye irritation, rabbit (OECD 405)	Non-irritant	Yes	None	None	[REDACTED] 1992 TOX9552441
IIIA 7.1.6/01 (B.5.11.1.6)	Skin sensitisation, guinea pig (OECD 406, Buehler (3 applications)	Non- sensitising	No	None	None	[REDACTED] 1992 TOX9552442
IIIA 7.1.6/02	Skin sensitisation, guinea pig (OECD 406, Buehler (9 applications)	Non- sensitising	Yes	None	None	[REDACTED] 2001 TOX2005- 1135
	Supplementary studies for combinations of plant protection products	No data – not required				

Summaries of previously reviewed studies (Sections 7.1.1 – 7.1.5) and the new dermal sensitisation study (IIIA 7.1.6/02) are presented below.

B.6.11.3 Acute oral toxicity

Reference: OECD IIIA 7.1.1
Report: [REDACTED] 1991
 Acute Oral Toxicity Study in Rats.,
 BD-91-261,
[TOX9552438](#)
Guidelines: US EPA FIFRA guideline 81-1 (1984)
 OECD 401 (1987),
 EEC directive 84/449/EEC method B.1 (1984)
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Test material (Lot/Batch No.) MON 52276 (LLN-9105-3135-F)
 Species Rat, Sprague-Dawley [CD[®] - CrI : CD[®] (SD)BR]
 No. of animals (group size) 5 males and 5 females
 Dose 5000 mg/kg bw
 Exposure Once by gavage
 Vehicle/Dilution None
 Post exposure observation period 14 days
 Remarks None

Results and discussions**Table B.6.11-5: Results of acute oral toxicity study in rats of MON 52276**

Dose (mg/kg bw)	Toxicological results ¹⁾	Duration of signs	Time of death	LD ₅₀ (mg/kg bw) (14 days)
Males				
5000	0/5/5	1 day	--	> 5000
Females				
5000	0/5/5	1 day	--	> 5000

¹⁾ Number of animals which died/number of animals with clinical signs/number of animals used

Table B.6.11-6: Summary of findings of acute oral toxicity study in rats of MON 52276

Mortality:	There were no mortalities during the study.
Clinical signs:	Faecal staining and / or soft stool was noted in all animals after dosing on day 1. A few animals also showed oral and / or nasal discharge, as well as hypoactivity.
Body weight:	Body weight gain was unaffected by the administration of the test substance.
Macroscopic examination:	The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Conclusion

Under the experimental conditions, the oral LD₅₀ of MON 52276 is higher than 5000 mg/kg bw in rats. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

B.6.11.4 Acute percutaneous toxicity

Reference: OECD IIIA 7.1.2
Report: [REDACTED] 1991
 Acute Dermal Toxicity Study in Rats.,
 BD-91-262,
[TOX9552439](#)
Guidelines: US EPA FIFRA guideline 81-2 (1984),
 OECD 402 (1987),
 EEC directive 84/449/EEC method B.3 (1984)
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Test material (Lot/Batch No.)	MON 52276 (LLN-9105-3135-F)
Species	Rat, Sprague-Dawley [CD [®] - CrI : CD [®] (SD)BR]
No. of animals (group size)	5 males and 5 females
Dose(s)	5000 mg/kg bw
Exposure	24 hours (dermal, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table B.6.11-7: Results of acute dermal toxicity study in rats of MON 52276

Dose (mg/kg bw)	Toxicological results ¹⁾	Duration of signs	Time of death	LD ₅₀ (mg/kg bw) (14 days)
Males				
5000	0/0/5	--	--	> 5000
Females				
5000	0/0/5	--	--	> 5000

¹⁾ Number of animals which died/number of animals with clinical signs/number of animals used

Table B.6.11-8: Summary of findings of acute dermal toxicity study in rats of MON 52276

Mortality:	There were no mortalities during the study.
Clinical signs:	There were no dermal effects observed in any of the animals throughout the study period. Two animals showed red ocular discharge and one additional animal had red-stained urine at day 1.
Body weight:	Body weight gain was unaffected by the administration of the test substance.
Macroscopic examination:	The gross necropsy conducted at termination of the study revealed no observable abnormalities.

Conclusion

Under the experimental conditions, the dermal LD₅₀ of MON 52276 is higher than 5000 mg/kg bw in rats. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

B.6.11.5 Acute inhalation toxicity

An acute inhalation toxicity study on MON 52276 has not been performed, because the criteria listed in Annex II (7.3.1) of Commission Regulation (EU) 545/2011 are not met (see below):

MON 52276 is not / does not

- a) a gas or liquified gas,
The pure active substance, glyphosate acid, is in the form of colourless crystals at ambient temperature, with a melting point of 189.5 °C. The preparation MON 52276
- is a soluble liquid (SL) formulation
- b) a smoke generating formulation or fumigant,
- c) used with fogging/misting equipment,
- d) a vapour releasing preparation,
The preparation is not a vapour releasing preparation. It is a soluble liquid (water-based), which is mixed with water for application by hydraulic sprayers
- e) an aerosol,
- f) a powder or a granule containing a significant proportion of particles of diameter < 50 µm (> 1 % on a weight basis), MON 52276 is a soluble liquid, not a powder
- g) to be applied from aircraft in cases where inhalation exposure is relevant,
- h) contain an active substance with a vapour pressure > 1x10⁻² Pa and is not to be used in enclosed spaces such as warehouses or glasshouses,
The active ingredient, glyphosate acid, is essentially non-volatile. Its vapour pressure is well below 1 x 10⁻² Pa., the threshold for consideration as a volatile substance:

Vapour pressure: 1.31 × 10⁻⁵ Pa (25 °C)

Henry's Law Constant: 2.1 × 10⁻⁷ Pa × m³ × mol⁻¹

Based on volatility, the calculated vapour density of glyphosate is less than 1 mg × m⁻³ at 25 °C (equivalent to less than 6 x 10⁻⁹ moles × m³).

In MON 52276, the active ingredient is formulated as the isopropylamine salt of glyphosate. The salt is less volatile than the acid:

Vapour pressure: 2.1 x 10⁻⁶ Pa (25 °C)

Henry's Law Constant: 4.6 x 10⁻¹⁰ Pa × m³ × mol⁻¹

The calculated vapour density of the isopropylamine salt of glyphosate is less than $0.2 \text{ mg} \times \text{m}^{-3}$ at 25°C (equivalent to less than $1 \times 10^{-9} \text{ moles} \times \text{m}^{-3}$).

- i) to be applied in a manner which generates a significant proportion (greater than 1% on a weight basis) of particles or droplets of diameter $< 50 \text{ }\mu\text{m}$ unless the applicant can justify an alternative approach under Directive 1999/45/EC or Regulation (EC) No 1272/2008, where applicable.

The product is recommended for spraying through hydraulic nozzles. Label recommendations propose that the nozzles used to atomise the spray mixture should produce a “medium” to “medium/coarse” spray quality as defined by the International (BCPC) spray classification system. Such nozzles produce a size range of droplets suitable to optimise their deposition on target weeds while reducing the proportion of droplets susceptible to drift.

Droplet spectra were measured for MON 52276 using standard nozzles typical for the type used on field sprayers in a supplementary study ([REDACTED] 1999; [ASB2012-12069](#)). The Spraying Systems 11003 nozzle used in the study is classified as producing a “fine/medium” spray and, therefore, represents a worst case in terms of the proportion of small droplets produced. The results for MON 52276 are comparable to those from studies on other formulations of glyphosate. The droplet size data are measured and reported as % volume, however, the specific gravity of a spray solution is close to 1.00.

Volume median diameter	246 μm	
Number median diameter	55 μm	
% total spray volume	$< 50 \text{ }\mu\text{m}$	0.71 %
	$< 10 \text{ }\mu\text{m}$	0.00 %

Less than 1 % (w/w) of the droplets have a smaller diameter than $50 \text{ }\mu\text{m}$, the threshold specified in Commission Regulation 545/2011 as a trigger to perform a mandatory acute inhalation toxicity study. Larger nozzles, such as Spraying Systems 11004, or “low drift nozzles”, produce fewer small droplets which would even represent a lower risk than those produced by standard nozzles.

B.6.11.6 Skin irritation

Reference: OECD IIIA 7.1.4

Report: [REDACTED] 1991
Primary Dermal Irritation Study in Rabbits.,
BD-91-263,
[TOX9552440](#)

Guidelines: OECD 404 (1992);
Commission Directive 92/69/EEC method B.4 (1992),
US EPA FIFRA guideline 81-5 (1984)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test material (Lot/Batch No.)	MON 52276 (LLN-9105-3135-F)
Species	Rabbit, New Zealand White
No. of animals (group size)	4 males and 2 females
Initial test using one animal	No
Exposure	0.5 mL (4 hours, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	3 days
Remarks	The test substance was applied on two sites of each animal (right and left).

Results and discussions

Table B.6.11-9: Skin irritation of MON 52276

Animal No.			Scores after treatment ¹⁾				Mean scores (24-72 h)	Reversible [day]
			0.5 h	24 h	48 h	72 h		
1 (male)	Right	Erythema	0	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	
2 (male)	Right	Erythema	1	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	2	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	
3 (female)	Right	Erythema	2	1	1	0	0.67	3
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	1	1	0	0.67	
		Oedema	0	0	0	0 ²⁾	0.0	
4 (male)	Right	Erythema	1	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	
5 (male)	Right	Erythema	1	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	
6 (female)	Right	Erythema	1	0	0	0	0.0	1
		Oedema	0	0	0	0	0.0	
	Left	Erythema	1	0	0	0	0.0	
		Oedema	0	0	0	0	0.0	

¹⁾ scores in the range of 0 to 4

²⁾ desquamation reported

Clinical signs::No mortality occurred. No clinical signs were reported.

Conclusion

Under the experimental conditions, MON 52276 is not a skin irritant. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

B.6.11.7 Eye irritation

Reference: OECD IIIA 7.1.5
Report: [REDACTED] 1992
Primary Eye Irritation Study in Rabbits,
BD-91-60,
[TOX9552441](#)
Guidelines: OECD 405 (1987);
EC Directive 92/69/EEC method B.5 (1992);
US EPA FIFRA guideline 81-4 (1984)
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

Test material (Lot/Batch No.)	MON 52276 (LLN-9102-2794-F)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 males and 3 females
Initial test using one animal	No
Exposure	0.1 mL (single instillation in conjunctival sac)
Irrigation (time point)	No
Vehicle/Dilution	None
Post exposure observation period	7 days
Remarks	None

Results and discussions

Table B.6.11-10: Eye irritation of MON 52276

Animal No.		Scores after treatment ¹⁾				Mean scores (24-72 h)	Reversible [day]
		1 h	24 h	48 h	72 h		
1 (female)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	1	1	1	0	0.67	3
	Chemosis conjunctivae	1	0	0	0	0.0	1
2 (male)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	1	1	1	0	0.67	3
	Chemosis conjunctivae	1	0	0	0	0.0	1
3 (female)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	2	0	0	0	0.0	1
	Chemosis conjunctivae	1	0	0	0	0.0	1
4 (male)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0.5	0	0	0	0.0	1
	Redness conjunctivae	2	2	2	1	1.7	7
	Chemosis conjunctivae	1	0	0	0	0.0	1
5 female)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	1	2	2	1	1.67	7
	Chemosis conjunctivae	1	0	0	0	0.0	0
6 (male)	Corneal opacity	0	0	0	0	0.0	0
	Iritis	0	0	0	0	0.0	0
	Redness conjunctivae	1	2	2	2	2.0	7
	Chemosis conjunctivae	1	0	0	0	0.0	1

¹⁾ scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae (1 is not considered positive by the applicants) and 0 to 2 (including 0.5 which is not considered positive) for iritis

Clinical signs: No mortality occurred. No clinical signs of systemic toxicity were reported.

Conclusion

Under the experimental conditions, MON 52276 is not an eye irritant. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

In addition to the toxicological study on rabbits an open publication was submitted by the applicants (Acquavella, J.F. et al., 1999; [TOX2002-699](#)) dealing with human ocular effects from self-reported exposure towards Roundup® herbicides. According to this publication no serious, long-lasting eye injury occurred during a period of 5 years among callers to a single regional poison center.

B.6.11.8 Skin sensitisation

For a summary of the non-GLP compliant study on skin sensitisation (IIIA 7.1.6/01, [REDACTED] 1992 ; [TOX9552442](#)) which was evaluated during the first Annex 1 inclusion procedure of glyphosate and which was not submitted for this renewal it is referred to the DAR.

Reference: OECD IIIA 7.1.6/02

Report: [REDACTED] 2001

Skin Sensitisation Test in Guinea Pigs (Modified Buehler test: 9 Applications),
CI-2001-153,
[TOX2005-1135](#)

Guidelines: OECD 406 (1992);

Commission Directive 96/54/EC B.6 (1996)

Deviations: No

GLP: Yes

Acceptability: Yes

Remark: The LLNA, or, if not possible, the M&K-test is clearly preferred to the Buehler-test according to the current state of knowledge and the expected data requirements for plant protection products for authorisation in the EU. According to REACH, the LLNA is the first choice method, too, and a justification for the use of a different test shall be provided. Test Method Guideline B.6 by the European Commission (Reg. (EC) No. 440/2008) or even by its previous version 96/54 also recommends the preferential use of an adjuvant-test (e.g. M&K-test) instead of the Buehler-test without adjuvant unless a justification is given for using the Buehler-method. However, no justification is available. But, since the provided Buehler-test is valid this is to be accepted against the background of animal welfare.

Materials and methods

Test material (Lot/Batch No.)	MON 52276 (A1C1204104)
Species	Guinea pig, Hartley CrI: (HA) BR
No. of animals (group size)	Test substance group: 10 male and 10 female guinea pigs Vehicle control group: 5 male and 5 female guinea pigs
Range finding	Yes
Exposure (concentration(s), no. of applications)	Topical induction: Undiluted (9 x) Challenge: Undiluted
Vehicle	Purified water
Pretreatment prior to topical application	No

Reliability check

Mercaptobenzothiazole (topical induction: 1st to 4th ind. 20 % w/w, 5th and 6th ind. 10 % w/w, 7th and 8th ind. 5 % w/w and 9th ind. 2.5 % w/w and challenge: 20 %)

Remarks

None

Results and discussions

Table B.6.11-11: Summary of skin responses after challenge exposure towards MON 52276

	24 hours	48 hours
	After challenge	
MON 52276	0/20	0/20
Control Group (Vehicle)	0/10	0/10
Positive control	2/10	7/10

¹⁾ Number of animals with positive dermal response (scores of 1-3)/number of animals in dose group

Clinical signs::No deaths occurred. No signs of systemic toxicity were reported.

Conclusion

Under the experimental conditions, MON 52276 is not a skin sensitiser. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

B.6.11.9 Supplementary studies for combinations of plant protection products

Combined application of plant protection products is not intended. Therefore, no such studies were performed.

B.6.12 Dermal absorption (Annex IIIA 7.6)

Introduction into this chapter by RMS

In the 2001 EU glyphosate evaluation, dermal absorption of glyphosate was considered to be less than 3% (DAR 1998, ASB2010-10302). This estimate was based on in vivo data in Rhesus monkeys, as well as on in vitro data in human skin, using the original glyphosate formulation Roundup (1983, TOX9552417; 1991, TOX9552418). However, these studies do not comply to current standards and should not be taken into consideration anymore even though the 3% assumption was supported by a new study with the active ingredient (2012, ASB2012-11459).

For exposure calculation and risk assessment, it is necessary to determine or estimate dermal absorption of an active ingredient from the formulation under evaluation since co-formulants may have a crucial impact on the absorption rate. Such studies are available for a small number of formulations containing glyphosate. For the representative formulation MON 52276, dermal absorption was assessed in vitro on human skin (isolated epidermis). Valid studies of this type are usually accepted in the EU as "stand alone-information" with their results (after appropriate rounding) being directly and without further adjustment used for exposure calculation and risk assessment (OECD, 2011, ASB2013-2; EFSA, 2012, ASB2012-

6959). Thus, the absence of *in vivo* data is not considered a data gap. This *in vitro* study (Ward, 2010, ASB2012-5383) and their results are reported in detail below.

Subsequently, a study is addressed in which dermal absorption of the active ingredient glyphosate itself (i.e., not contained in a commercial formulation) was investigated *in vitro* on rabbit skin (Hadfield, 2012, ASB2012-11459). Even though rabbit skin is a rather unusual model for studies of this type, the study is reported because it may give an idea of the low dermal absorption of glyphosate that is not formulated to generate a specific plant protection product.

Four more *in vitro* dermal absorption studies were submitted as part of the GTF dossier (Davies, 2003, ASB2012-11518; Ward, 2010, ASB2012-11515; Ward, 2010, ASB2012-11516; Hadfield, 2012, ASB2012-11517) that all confirmed a low dermal absorption of glyphosate. However, since these studies were performed with products other than the representative formulation, there is no need to take them into consideration for the current re-evaluation of glyphosate. Accordingly, they were not reviewed by the RMS and have been excluded from Volume 3. If necessary, they should be evaluated in future on national or zonal level.

B.6.12.1 Dermal absorption of glyphosate from MON 52276 *in vitro* (Human epidermis)

Reference: IIIA, 7.6.2/01

Report: Ward, R.J. 2010 360 g/L Glyphosate SL Formulation (MON 52276) – *In vitro* absorption of Glyphosate through human epidermis
Dermal Technology Laboratory Ltd., Med IC4, Keele University
Science and Business Park, Keele, Staffordshire, ST5 5NL, UK
Study No.: JV2084, Report No.: JV2084-REG,
Date: 2010-02-19, Unpublished
ASB2012-5383

Guidelines: OECD 428

Deviations: None

GLP: yes

Acceptability: See RMS comment

Dates of experimental work: 2009-06-09 - 2009-08-26

The objective of this study was to evaluate the potential dermal absorption of glyphosate from a 360 g/L SL formulation concentrate, as well as from two representative in-use dilutions prepared as 1:12.5 (v/v) and 1:150 (v/v) aqueous dilutions.

¹⁴C-glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The doses were applied to human epidermal membranes at a rate of 10 µL/cm² and left unoccluded for an exposure period of 24 hours. The absorption process was followed by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout the exposure period. The distribution of glyphosate within the test system and a 24-hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).

Material and methods

Test materials:

Non radio-labelled test substance:

Identification: Isopropylamine salt of glyphosate techn. material (glyphosate-IPA)
 Description: Clear, water white to amber viscous liquid (solution in water)
 Lot/Batch #: A8B60170S0
 Chemical purity/a.i. content: Glyphosate-IPA: 63.81 %
 Glyphosate acid: 47.28 %
 Stability of test compound: Expiry date: 2012-01-25

Analytical reference standard:

Identification: Glyphosate acid
 Description: White solid
 Lot/Batch #: GLP-0810-19515-A
 Chemical purity: 99.8 %
 Stability of test compound: Expiry date: 2011-01-30

Radio-labelled test substance

Identification: ^{14}C -glyphosate (as glyphosate acid)
 Lot/Batch #: 53463-3-23
 Chemical purity: 99.8 %
 Radiochemical purity: 97.8 % (confirmed by analysis)
 Specific activity: 47 mCi/mmol; 1739 MBq/mmol; 277.9 $\mu\text{Ci/mg}$; 10.28 MBq/mg
 Stability of test compound: Not reported

Blank formulation

Identification: Proprietary surfactant blend (MON 8153)
 Concentration of a.s.: 0 %
 Description: Not reported
 Lot/Batch #: Not reported

Stability of test compound: Not reported

Formulated test substance

Identification: MON 52276
 The formulation concentrate used was not supplied as complete formulation, but had to be prepared from the ingredients a) and c) described above, to allow the incorporation of the radiolabel.
 The test substance concentration in the prepared formulation was confirmed by analysis.

Test skin source:

Species: Human

Source: Tissue bank (not further specified)

Study design and methods

Preparation of skin samples: Human skin samples (details regarding the donors not given) were immersed in water at 60 °C for 40-45 seconds and the epidermis was teased away from the dermis. Each membrane was given an identifying number and stored frozen at approximately -20 °C, on aluminium foil until required for use.

Test substance preparation

Three test substance concentrations representing the formulation concentrate and two field dilutions were prepared at target concentrations of 360 g/L, 29.6 g/L and 2.51 g/L. The nominal radioactivity contained in the dose preparations was 3.3 MBq.

Radioactive stock solution of ^{14}C -glyphosate

Dry ^{14}C -glyphosate was solubilised in 2 mL of water and mixed thoroughly.

High dose (formulation concentrate, 360 g/L)

A pre-mix was prepared by mixing 3900 mg glyphosate-IPA technical material with an appropriate amount of proprietary surfactant blend. 78 μL (\equiv 78 mg) of the radioactive stock solution was mixed with 482 mg of the pre-mix. Water was added to give a total weight of 585 mg. The solution was mixed well. Assuming a density of 1.17 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 360 g glyphosate/L.

Intermediate dose, (1:12.5 (v/v) aqueous dilution, 29.6 g/L)

A pre-mix was prepared by mixing 305.92 mg glyphosate-IPA technical material with an appropriate amount of proprietary surfactant blend. 78 μL (\equiv 78 mg) of the radioactive stock solution was mixed with 38.01 mg of the pre-mix. Water was added to give a total weight of 500 mg. The solution was mixed well. Assuming a density of 1 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 29.6 g glyphosate/L.

Low dose (1:150 (v/v) aqueous dilution, 2.51 g/L)

A pre-mix was prepared by mixing 76.90 mg glyphosate-IPA technical material with an appropriate amount of proprietary surfactant blend. 78 μL (\equiv 78 mg) of the radioactive stock solution was mixed with 2.64 mg of the pre-mix. Water was added to give a total weight of 500 mg. The solution was mixed well. Assuming a density of 1 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 2.51 g glyphosate/L.

Analyses of dose preparations

The radioactivity content of the stock solution was determined by liquid scintillation counting (LSC) analyses of sub-samples of solvent dilutions. The radiochemical purity of the radiolabelled test substance was determined by high performance liquid chromatography (HPLC).

The radioactivity content and homogeneity of the dose preparations were checked by LSC analyses. The radiochemical purity and stability was measured by HPLC analyses.

Preparation of diffusion cells

The skin membranes were placed in static glass diffusion cells providing an exposure area of 2.54 cm² of skin. The cells had a receptor chamber volume of approximately 4.5 mL.

An integrity test was performed by measuring the electrical resistance across the skin membranes. Membranes with a resistance of ≥ 10 k Ω were considered having a normal integrity and used for the absorption study.

Physiological saline was chosen as receptor fluid. The skin surface temperature was maintained at 32 ± 1 °C using a water bath.

Test substance application and sampling

Prior to dosing a pre-treatment sample of 500 µL was taken from each diffusion cell, and replaced by an equal amount of fresh receptor fluid.

Each dose formulation was applied to the skin membrane at the rate of 10 µL/cm² exposed skin area (25.4 µL dose), corresponding to target concentration of 3693 µg/cm², 296 µg/cm² and 25.1 µg/cm² for the high, intermediate and low dose level, respectively. The applications were left un-occluded for 24 hours.

Receptor fluid samples (500 µL) were taken by an auto-sampler at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. After each sampling the removed amount of receptor fluid was replaced by an equal amount of fresh receptor fluid.

Terminal procedures

After the last sampling 24 hours after application, the remaining receptor fluid was discarded. The receptor chamber was rinsed with receptor fluid that was also discarded.

The donor chamber was carefully removed and the underside wiped with a single natural sponge, pre-wetted with 3 % Teepol L® in water, which was added to the wash sponges. The donor chamber was washed with deionised water and a sample was taken for LSC analysis.

The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The skin surface was washed with further sponges pre-wetted with water. All the sponges were combined and digested in Soluene 350® and made up to a recorded volume. A sample was taken for analysis.

The surface of the skin was allowed to dry naturally

Each skin membrane was tape stripped using 3M Scotch 'Magic' tape to a maximum of five strips. The tape strips were soaked individually in 30 % v/v methanol in water to extract any test material. The extracts were sequentially numbered and analysed by LSC. In some cases, it was not possible to take the full five tape strips as the epidermis began to tear, therefore tape stripping was discontinued. The last tape strip for these diffusion cells was digested with the remaining epidermis, so as not to underestimate residues in the remaining epidermis compartment. The remaining epidermis was carefully removed from the receptor chamber and digested in Soluene 350® and the whole digest analysed by LSC.

Analysis of samples

Liquid samples of the receptor fluid, washing solutions, digested wash sponges, tape strip extracts and digested epidermis by LSC using a Packard 3100 TR LSC counter and Goldstar as scintillation fluid.

Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of glyphosate in the receptor solution in terms of µg/cm². The amounts absorbed, rates of absorption (µg/cm²/h) and 'percentage of dose absorbed' were calculated. Membranes with absorption profiles that indicate membrane damage during the course of the experiment have been excluded from calculations. The results of the mass balance and distribution determinations are expressed in terms of amount absorbed and 'percentage of applied dose'.

The absorbed dose is considered the glyphosate detected in the receptor fluid, while the potentially biologically available proportion of the dose is regarded as the sum of absorbed dose and the amount recovered from the epidermis after tape stripping. The test material

removed from the surface of the epidermis by the washing procedure, as well as the glyphosate recovered from the epidermis at the end of the exposure is considered unabsorbed.

Results and discussion

Analysis of unformulated ^{14}C -glyphosate

HPLC analysis of the unformulated sample of ^{14}C -glyphosate confirmed a radiochemical purity of 97.8 %.

Analyses of dose preparations

The achieved concentration of glyphosate in the dose preparations was calculated to be 369.3, 29.6 and 2.52 g glyphosate /L in the formulation concentrate, 1/12.5 v/v dilution and 1/150 v/v dilution, respectively.

LCS analyses confirmed the dose solutions to be homogeneous.

Dermal absorption of glyphosate

The determined distribution of radioactivity for the different dose groups are summarised in Table B.6.12-1 below.

Table B.6.12-1: Summary of results for dermal absorption of ^{14}C -glyphosate from representative SL formulation MON 52278

Dose preparation	High (concentrate)		Intermediate (1:12.5 v/v dilution)		Low (1:150 v/v dilution)	
Nominal concentration [g/L]	360		29.6		2.51	
Actual concentration [g/L]	369.3		29.6		2.52	
Applied dose [$\mu\text{L}/\text{cm}^2$]	10		10		10	
Applied dose [$\mu\text{g}/\text{cm}^2$]	3693		296		25.2	
Number of cells accessed	4*		6		4*	
	Distribution of radioactivity (mean values)					
	$\mu\text{g}/\text{cm}^2$	% of applied dose	$\mu\text{g}/\text{cm}^2$	% of applied dose	$\mu\text{g}/\text{cm}^2$	% of applied dose
<i>Surface compartment</i>						
Stratum corneum (5 tape strips)	2.39	0.065	0.386	0.130	0.081	0.320
Stratum corneum (first 2 tape strips)	1.57	0.043	0.283	0.096	0.065	0.256
Skin wash	3656	99.0	288	97.4	24.8	98.4
Donor chamber	83.4	2.26	6.67	2.26	<LOQ	0.008
<i>Receptor compartment</i>						
Receptor fluid (0-24 h)	0.322	0.009	0.086	0.029	0.023	0.092
Total absorbed	0.322	0.009	0.086	0.029	0.023	0.092
Remaining epidermis (after 5 tape strips)	2.02	0.055	0.310	0.105	0.047	0.185
Remaining epidermis (after 2 tape strips)	2.84	0.077	0.413	0.140	0.063	0.250
Total potentially absorbable** (after 5 tape strips)	2.343	0.063	0.396	0.134	0.070	0.276
Total potentially absorbable** (after 2 tape strips)	3.162	0.086***	0.499	0.169	0.086	0.342***
Total recovery	3744	101	296	100	25.0	99.0
Absorption rates [$\mu\text{g}/\text{cm}^2/\text{h}$] (0-24h)	0.014		0.003		0.001	

* Some cells for these applications were excluded from calculations as the analytical data indicated that the epidermal membrane may have been damaged during application.

** Total potentially absorbable = total absorbed + remaining epidermis

*** Dermal absorption values used for exposure assessment

The overall total recovery for the three dose levels was good, with mean values of 99 – 101 % of the applied dose.

Glyphosate absorption from the 360 g/L concentrate formulation was essentially constant over the entire 24 hour exposure period (mean rate = 0.014 µg/cm²/h). By the end of the exposure period, the mean total amount of absorbed glyphosate was 0.322 µg/cm² (0.009 % of applied dose).

From the intermediate and low-dose aqueous dilutions of the formulation, absorption was fastest during the early period of absorption, with 0.010 µg/cm²/h, (0-1h) and 0.004 µg/cm²/h, (0-2h), respectively. The rates after this early period until the end of the exposure at 24h were 0.003 µg/cm²/h and 0.001 µg/cm²/h for the intermediate and low dose dilutions, respectively. At the end of the exposure period, the mean total amounts of absorbed glyphosate were 0.086 and 0.023 µg/cm² (0.029 % and 0.092 % of applied dose), respectively.

For the formulation concentrate and both aqueous dilutions, the vast majority of the applied glyphosate was removed from the surface of the epidermis during the washing procedure at the end of the 24-hour exposure period (mean 97.4-99.0 %). The mean total amount of glyphosate recovered from the epidermis was 0.120 %, 0.235 % and 0.505 % of the applied dose for the concentrate, intermediate and low dose dilution, respectively.

The amount of potentially biologically available glyphosate (absorbed + epidermis after tape stripping) for the concentrate, intermediate and low dose dilutions were 0.064 %, 0.134 % and 0.277 % respectively for 5 tape strips and more conservatively 0.086 %, 0.169 % and 0.342 % respectively for only 2 tape strips.

Conclusions by the Notifier

The results of this *in vitro* dermal absorption study indicate that the absorption of glyphosate through human skin is very limited and very slow. The vast majority of glyphosate was removed from the skin by the washing procedures.

The total absorbed amounts after 24 hour exposure were 0.009 %, 0.029 % and 0.092 % of the applied dose for the formulation concentrate, the 1:12,5 (v/v) and 1:150 (v/v) dilution, respectively.

The corresponding total potentially absorbable amounts, represented by the mean absorbed dose together with the amounts in the remaining skin were 0.063 %, 0.134 % and 0.276 %, respectively for 5 tape strips and more conservatively 0.086 %, 0.169 % and 0.342 % respectively for only 2 tape strips.

Thus, the results predict that the dermal absorption of glyphosate from potential exposure to this 360 g/L glyphosate / L SL formulation would be less than 1 %, irrespective of whether two or five tape strips were considered to contain non-biologically available glyphosate.

Table B.6.12-2: Summary of glyphosate dermal absorption from MON 52276

Study	% of applied dose*			Reference
	Concentrate	Spray dilutions		
SL formulation	360 g/L	29.6 g/L	2.51 g/L	
<i>In vitro</i> (human skin)	0.086	0.169	0.342	IIIA 7.6.2 Ward, 2010a

* The absorption values correspond to total amounts potentially absorbable through human skin (i.e. amounts of radioactivity recovered in the receptor fluid and remaining skin after tape stripping with two strips).

RMS comments

The study is considered acceptable. The mass balance was very good and any adjustment for test material losses not needed. Very low dermal absorption of glyphosate from the representative formulation has been shown. It must be emphasised that the use of isolated epidermis (instead of dermatomed skin) and an exposure period of 24 hours will most probably result in overestimation of the dermal absorption rate.

The approach taken by the applicant to calculate the dermal absorption is agreed with. In line with current EU practice and also with the new European Guidance document on dermal absorption (EFSA, 2012, ASB2012-6959) the first (upper) tape strips were excluded. The amount retained in the lower (three) tape strips, in contrast, should be considered potentially absorbable because raw data have shown that 75 % penetration (expressed as the percentage of radioactivity in the receptor fluid) was not achieved for any of the three applied concentrations after one half of the study duration, i.e., after 12 hours. The achieved mean values were rounded according to new rules (EFSA, 2012, ASB2012-6959) giving dermal absorption rates of 0.1, 0.2, and 0.3 % for the high, mid and low concentration that should be used for exposure calculation and risk assessment purposes. The RMS is aware that the new European Guidance document has come into force long after conduct of the study and also after submission of the glyphosate dossier. Thus, in a strict sense, it might not be applicable. However, it was used here to support the approach taken with regard to inclusion/exclusion of tape strips and for rounding in the hope that it might be helpful for a harmonised evaluation of this study.

B.6.12.2 Dermal absorption of glyphosate active substance *in vitro* (abraded rabbit skin)

Reference: IIA, 5.3.7/02
Report: Hadfield, N. 2012a Glyphosate acid - *In Vitro* Absorption through Abraded Rabbit Skin using [¹⁴C]-glyphosate
 Dermal Technology Laboratory Ltd., Med IC4, Keele University
 Science and Business Park, Keele, Staffordshire, ST5 5NL, UK
 Study No.: JV2182, Report No.: JV2182-REG
 Date: 2012-04-18, Unpublished,
[ASB2012-11459](#)
Guidelines: OECD 428
Deviations: None
GLP: yes
Acceptability: See RMS comment

Dates of experimental work: 2011-12-12 to 2011-12-22

The purpose of this study was to determine the *in vitro* percutaneous absorption of glyphosate acid through abraded rabbit skin following a 6-hour exposure period and subsequent 18 hour monitoring period. This study was designed to assess the potential dermal penetration of test material through rabbit skin and will be of use in estimating the systemic dose achieved in a previous *in vivo* rabbit dermal toxicity study (see IIA 5.3.7, Johnson, 1982, TOX9552366). Therefore, the application rate and exposure conditions used in this study were calculated to be equivalent to 5000 mg/kg bw/day as applied to rabbits in the *in vivo* dermal study (IIA 5.3.7).

¹⁴C-glyphosate was incorporated into a wet cake preparation prior to application. The preparations were applied as a paste to abraded rabbit skin membranes at a rate of

79.8 mg/cm² (corresponding to 48.3 mg glyphosate acid/cm²) and left unoccluded for an exposure period of 6 hours, after which the skin surface was washed. The absorption process was followed by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout a total time-period of 24 hours. The distribution of glyphosate within the test system and a 24-hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).

Materials and methods

Test materials:

Non radio-labelled test substance:

Identification: MON 77973 (glyphosate acid)
 Description: White wet cake
 Lot/Batch #: GLP-1103-21149-T
 Chemical purity/a.i. content: 85.14 % as glyphosate acid (purity: 95.93 %)
 Stability of test compound: Expiry date: 2012-03-09
 Analytical reference standard:

Identification: Glyphosate acid
 Lot/Batch #: GLP-0810-1915-A

Chemical purity: Not reported

Radio-labelled test substance

Identification: ¹⁴C-glyphosate (as glyphosate acid)
 [phosphonomethylene-¹⁴C]-glyphosate
 Lot/Batch #: 4675JJN002-1
 Radiochemical purity: 96.7 % (confirmed by analysis)
 Specific activity: 48 mCi/mmol; 1776 MBq/mmol; 2523 µCi/mL; 9.35 MBq/mL

Test skin source:

Species: Rabbit
 Strain: New Zealand White Albino
 Source: Harlan
 Age: At least 12 weeks
 Type: Complete pelt

Study design and methods:

Preparation of skin samples

Skin pelts from New Zealand White albino rabbits at least 12 weeks old were obtained from Harlan. The skin samples were transported on cold blocks and were stored on arrival at -20°C, the day after sacrifice. The skin samples arrived clipped and excised and were examined for scars and blemishes. Any extraneous subcutaneous tissue was removed after defrosting and the pelts clipped further if necessary. The pelts were given an identifying number and individually stored frozen, at approximately -20°C, on aluminium foil until required for use.

Test substance preparation

The doses were prepared, to mimic as closely as possible a 5000 mg/kg dose from a previous rabbit *in vivo* study (IIA 5.3.7, [REDACTED] 1982, TOX9552366). The dose equivalency was calculated on a dose per unit area of skin basis using an average *in vivo* rabbit weight of 2.78 kg. The doses were prepared as close to the time of application as was practicable.

Radioactive stock solution of ^{14}C -glyphosate

The radiolabelled ^{14}C -glyphosate was supplied as a solution in water.

Trial preparation of the radiolabelled glyphosate acid

Glyphosate acid trial preparation was prepared using the method described below, with the exception that different volumes or smaller amounts of radioactivity or unlabelled material were used, where applicable. Three individual vials were prepared as part of the trial preparation, to assess dosing methodology. The paste like composition of the dose preparation was investigated to ensure that it visually provided good skin contact during application to the membranes.

Preparation of radiolabelled glyphosate acid

Firstly 8008 mg of non-labelled glyphosate wet cake was added to a vial, followed by 4162 μL of radiolabelled glyphosate stock solution, providing a nominal 3.85 mg of glyphosate (40 MBq) radioactivity. 5 mL of water was then added and the preparation mixed thoroughly. The preparation was then freeze dried to remove the water added and the water present in the wet cake. When dry, the glyphosate wet cake preparation was then weighed to confirm the removal of all the water. Approximately 521 mg of the dried wet cake preparation was then added to 8 individual vials together with approximately 300 μL of saline to each vial to create a paste. A final weight of each vial was recorded and the preparation was thoroughly mixed with a spatula into a paste before dosing.

Preparation of non-labelled glyphosate acid

To demonstrate that the dose preparations have a close contact during the application procedure, an additional dose preparation without radiolabel was prepared according to the procedure described above.

Analyses of dose preparations

The radioactivity content of the stock solution was determined by liquid scintillation counting (LSC) analyses of sub-samples of solvent dilutions. The radiochemical purity of the radiolabelled stock solution of the test substance was determined by thin layer chromatography (TLC) using unlabelled test substance as reference standard.

The radioactivity content and homogeneity of the dose preparations were checked by LSC analyses. The radiochemical purity and stability was measured by TLC analyses.

Preparation of diffusion cells

The skin membranes were placed in static glass diffusion cells providing an exposure area of 2.54 cm^2 of skin. The cells had a receptor volume of approximately 4.5 mL.

An integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. Non-abraded membranes with a resistance of 1.5 - 5 $\text{k}\Omega$ were considered having a normal integrity and used for the skin abrasion. Rabbit skin was abraded using a blunt spatula drawn over the skin area approximately six to eight times, in the form of a grid, in order to mimic 'Draize' abrasion as conducted in the *in vivo* study (IIA 5.3.7, [REDACTED] 1982, TOX9552366). After the abrasion a further integrity test was performed by measuring the

electrical resistance (ER) across the skin membranes. For abraded skin samples membranes with ER values in the range of 0.7 – 1.0 k Ω were selected for the study.

Cells were selected such that the application rate was represented by eight intact skin samples from five different animals. Physiological saline was chosen as receptor fluid. The skin surface temperature was maintained at 32 ± 1 °C using a water bath.

Test substance application and sampling

Prior to dosing a pre-treatment sample of 500 μ L was taken from each diffusion cell, and replaced by an equal amount of fresh receptor fluid.

Each dose formulation was applied to the abraded skin membrane as a dried glyphosate acid wet cake paste and spread over the skin surface using a spatula. The weight of each individual preparation and spatula were recorded before and after dosing to allow the applied dose to be calculated.

Each dose was applied at the nominal rate of 79.8 mL/cm² exposed skin area (202.8 mg/cell), corresponding to 48.3 mg glyphosate/cm². The applications were left un-occluded for 24 hours.

Receptor fluid samples (500 μ L) were taken by an auto-sampler at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. After each sampling the removed amount of receptor fluid was replaced by an equal amount of fresh receptor fluid.

After the 6-hour sampling, the skin samples were washed by gently swabbing the application site application site with at least three natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. Two further sponges, pre-wetted with water, were used to further swab the surface.

Terminal procedures

After the last sampling, 24 hours after application the remaining receptor fluid was discarded. The receptor chamber was rinsed with receptor fluid that was also discarded.

The donor chamber was carefully removed and the underside wiped with a single natural sponge, pre-wetted with 3 % Teepol L® in water, which was added to the wash sponges. The donor chamber was washed with deionised water and a sample was taken for LSC analysis.

The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The skin surface was washed with further sponges pre-wetted with water. All the sponges were combined and digested in Soluene 350® and made up to a recorded volume. A sample was taken for analysis.

Due to the fragility of the abraded skin samples tape stripping could not be performed. Instead a heat separation technique was used to separate the epidermis from the dermis.

The skin was carefully removed from the receptor chamber and the flange area cut away and digested in Soluene 350® and aliquots taken for analysis by LSC.

The remaining skin disc was placed dermis side down, on cling film. A second piece of cling film was then used to cover the epidermis side. A 200g weight was placed in a water bath at 65°C for an hour prior to use. The weight was placed onto the epidermal surface with moderate pressure for approximately 90 seconds. The epidermis was peeled away from the dermis using forceps. The dermis was digested in Soluene 350® and aliquots taken for

analysis by LSC. The epidermis was digested in Soluene 350® and the whole sample analysed by LSC.

Analysis of samples

The radiochemical purity and stability of the ^{14}C -glyphosate preparations was determined by TLC using silica gel plates and methanol : water : acetic acid (6 : 3 : 0.5, v/v/v). Radioactivity on the TLC plates were measured using a Packard Instant Imager (SOP E003). Unlabelled material was visualised under UV light at 254 nm.

For visualising the test material on the TLC plates a 2 % ninhydrine solution in acetone was used.

In addition, for analyses of dose preparations K2 cellulose plates and a revised solvent system (methanol : water : acetic acid (8 : 1.5 : 0.5, v/v/v) was used.

Liquid samples of the receptor fluid, washing solutions, digested wash sponges, and digested dermis and epidermis were measured by LSC using a Packard 3100 TR LSC counter and Goldstar as scintillation fluid.

Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of glyphosate in the receptor solution in terms of $\mu\text{g}/\text{cm}^2$. The amounts absorbed, rates of absorption ($\mu\text{g}/\text{cm}^2/\text{h}$) and 'percentage of dose absorbed' were calculated. Membranes with absorption profiles that indicate membrane damage during the course of the experiment have been excluded from calculations. The results of the mass balance and distribution determinations are expressed in terms of amount absorbed and 'percentage of applied dose

The absorbed dose is considered the glyphosate detected in the receptor fluid, while the potentially biologically available proportion of the dose is regarded as the sum of absorbed dose and the amount recovered from the dermis. The test material removed from the surface of the epidermis by the washing procedure, as well as the glyphosate recovered from the epidermis at the end of the exposure is considered unabsorbed.

Results and discussion

Analyses of the ^{14}C -glyphosate stock solution

TLC analysis of the ^{14}C -glyphosate stock solution confirmed a radiochemical purity of greater than 95 %.

LSC analysis revealed a radioactivity content of 72.1 MBq, equivalent to a concentration of 0.924 mg/mL. The stock solution was homogeneous with a 1.31 % deviation between the replicates.

Analyses of dose preparations

LSC analyses confirmed the mean application rate to be 48.3 mg glyphosate/ cm^2 .

The dose preparations had low variability between the replicates analysed (1.66 %-6.26 %) and, considering the physical nature of the preparation, the dose preparations were considered to have acceptable homogeneity.

Membrane integrity check

Based on the ER measurements eight cells with abraded skin samples were selected for the absorption study.

Dermal absorption of glyphosate

Absorption profiles were assessed from eight abraded skin samples. Since one skin sample showed an atypical absorption profile, this was excluded from the calculation of means and SD.

The determined distribution of radioactivity are summarised in Table B.6.12-3 below.

Table B.6.12-3: Summary of results for dermal absorption of ^{14}C -glyphosate (rabbit skin)

Dose preparation				
Applied dose "wet cake" [mg/cm^2]	79.8			
Applied dose glyphosate [mg/cm^2]	48.3			
Number of cells assessed	7			
	Distribution of radioactivity (mean values)			
	$\mu\text{g}/\text{cm}^2$	SD	% of applied dose	SD
<i>Surface compartment</i>				
Dermis (after heat separation)	118	19.4	0.243	0.040
Skin wash at 6 hours	42802	3008	87.9	6.30
Skin wash at 24 hours	1159	1224	2.38	2.51
Donor chamber	59.2	56.9	0.121	0.117
<i>Receptor compartment</i>				
Receptor fluid (0-24 h)	1177	244	2.42	0.503
Total absorbed*	1177	--	2.42	--
Epidermis (after heat separation)	20.1	9.97	0.041	0.020
Flange area	132	68.6	0.270	0.141
Total potentially absorbable**	1295	--	2.663	--
Total recovery	45468	2096	93.3	4.46
Absorption rates [$\mu\text{g}/\text{cm}^2/\text{h}$] (0-24h)	53.1	10.2	--	--

SD Standard deviation

* Amount in receptor fluid.

** Total potentially absorbable = total absorbed + remaining dermis (after heat separation)

The total recovery of the individual cells was in the range of 87.3 % to 98.2 %, with an overall mean recovery of 93.3 % of applied dose.

The majority of the applied glyphosate acid (mean 87.9 %) was washed off the skin at 6 hours, with a further 2.38 % washed off at 24 hours. A small proportion (0.041 %) of the dose applied was recovered from the epidermis, with 0.243 % remaining in the dermis.

The mean rate of absorption of glyphosate acid between 0-1 hours was $47.0 \mu\text{g}/\text{cm}^2/\text{h}$, which increased to $166 \mu\text{g}/\text{cm}^2/\text{h}$ between 1-4 hours. The mean absorption rate subsequently slowed to $72.3 \mu\text{g}/\text{cm}^2/\text{h}$ between 4-10 hours and declined further to $13.3 \mu\text{g}/\text{cm}^2/\text{h}$ for the remainder of the absorption period (10-24 hours). The overall absorption rate (0-24 hours) was $53.1 \mu\text{g}/\text{cm}^2/\text{h}$.

The mean amount of glyphosate acid that penetrated abraded rabbit skin into the receptor fluid over the entire 24-hour experimental period was $1177 \mu\text{g}/\text{cm}^2$, corresponding to 2.42 % of the applied dose.

Considering that the amount found in the remaining dermis after 24 h is potentially available and could further penetrate through the skin, the total amount of glyphosate potentially available was 2.66 % of the applied dose

Conclusions by the Notifier

The results of this *in vitro* study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow. The vast majority of glyphosate will be washed off during normal washing procedures. The mean total amount absorbed after 24 hours was 2.42 %. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 %.

RMS comments

The study is considered supplementary despite its good quality. Using abraded rabbit skin as an in vitro model, it could be shown that dermal absorption of glyphosate active ingredient was less than 3 %, confirming to some extent a previous assumption (DAR 1998, ASB20110-10302) that was based on data obtained in Rhesus monkeys in vivo (██████████ 1991, TOX9552418). However, even though the information provided by this study is interesting from a purely scientific point of view, its regulatory value will be quite limited. Dermal absorption of an active ingredient must be always determined or estimated for a certain formulation under evaluation. Exposure to glyphosate in a "wet cake" in this study does neither reflect real exposure conditions of operators, bystanders or workers to liquid concentrates or spray dilutions nor a possible impact of co-formulants.

B.6.12.3 Publications on dermal absorption of glyphosate

Wester *et al.* (2005, ASB2012-12050) compared dermal absorption through human skin *in vitro* after exposure either to a 1 % solution (not further specified) or to cotton sheets that were treated with the same solution on the same day or up to 2 days before to reflect the abundance of glyphosate residues in fabric that may occur due to applications of this active ingredient in cotton. The authors could show a lower absorption of glyphosate across the skin from the treated cotton as compared to the liquid solution itself but found also remarkable residues on and in the skin when the cotton sheets had been treated on the same day. Adding of water to the cotton sheets resulted in an increase in absorption rate.

B.6.13 Toxicological data on non active substances (Annex IIIA 7.9 and point 4 of the introduction)

B.6.13.1 Material safety data sheet for each formulant

Copies of the safety data sheets of the formulants are provided in Document J of this dossier.

B.6.13.2 Available toxicological data for each formulant

Please refer to the material safety data sheets provided in Document J of this dossier. There is no toxicological classification of co-formulants which has to be considered for classification and labelling of MON 52276 according to these MSDS.

Remarks on surfactants included into glyphosate-containing plant protection products

All glyphosate-containing plant protection products contain surfactants or - if not present as an integral component – are to be mixed with surfactants as a compulsory additive to produce the ready-to-use dilution. As has already been discussed during the first Annex I inclusion procedure for glyphosate it became apparent that glyphosate-containing products were more toxic than glyphosate alone. This phenomenon was attributed to the presence of particular surfactants predominantly, namely the POE- [REDACTED]

Some MS may wish to allow for this in the context of the national risk assessment for [REDACTED] containing glyphosate formulations. Therefore, a toxicological evaluation for POE- [REDACTED] (including reference values) is provided in a separate paragraph within Vol. 3 (B.6.13.3) of this RAR.

MON 52276 which is the representative formulation here does not contain any [REDACTED]. Instead, a different type of surfactant, i.e. a quarternary ammonium compound, is used for MON 52276.

Since studies on MON 52276 concerning acute toxicity, skin and eye irritation as well as skin sensitisation were performed with the original preparation of MON 52276 the results for these toxicological short-term endpoints also reflect possible effects provoked by the surfactant. No further studies are needed according to the data requirements for plant protection products. Therefore, no toxicological long-term studies were submitted using the formulated product or the surfactant alone. Moreover, up to now no reference values have been considered necessary for the surfactant used, thus, no respective risk assessment was required.

According to the material safety data sheet for the surfactant provided by the applicants this co-formulant was not mutagenic in an Ames-test. No further information on toxicological long-term endpoints was given in this material safety data sheet.

In addition, MON 52276 has been authorised within the EU for many years. There are no medical data which have been collected by occupational physicians or poisoning emergency centres describing long-term adverse health effects for operators provoked by this plant protection product until today.

B.6.13.3 Further toxicological data for other potential co-formulants

The following toxicological evaluation of [REDACTED] surfactants was prepared by the German Federal Institute for Risk Assessment in 2010 but was not discussed on EU level so far. Even though it is not essential for current risk assessment of glyphosate, i.e., re-evaluation under Regulation (EC) 1107/2009, it might facilitate product assessment and authorisation on zonal or MS level. Therefore, it has been included here as an appendix. Some of the references mentioned in this evaluation have been also submitted in the GTF dossier on glyphosate but had been provided on request to the RMS before or were found as result of a separate literature search. New references were not included and the text of the 2010 evaluation itself was not amended for other than editorial reasons (mainly correction of typos and citation of references). In rare cases, references to other parts of the current RAR were made. The only exception from this approach was dermal absorption that is now estimated by using the default values of 25 and 75 % as recommended in the EU guidance (EFSA, 2012, ASB2012-6959). Enumeration of tables was kept.

B.6.13.3.1 Toxicological evaluation of the POE-[REDACTED] surfactant (CAS no. 61791-26-2)

Objective of this evaluation:

Many plant protection products (PPP), in particular a number of (but not all) formulations of the widely used herbicidal active ingredient glyphosate, contain a substance with the CAS no. 61791-26-2 as a surfactant. This substance (sum formula, according to U.S.EPA, $C_{48}H_{97}NO_{15}$) is also known as "POE-[REDACTED]" and belongs to the heterogeneous chemical group of polyethoxylated alkylamines (POEA). A huge amount of information from different sources (poisoning incidents in humans; *in vitro* data obtained in different test systems; studies on short-term, reproductive and developmental toxicity of Roundup® formulations or preparations), even though of different quality and reliability, in the whole suggests a higher toxicity of such PPP as compared to the active compound. Glyphosate itself is generally considered to be of low toxicological concern ([REDACTED] 2000, ASB2012-12053; EU, 2001, ASB2009-4191; JMPR, 2004, ASB2008-6266). However, in the DAR on glyphosate (DAR, 1998, ASB2010-10302) that was prepared to support first Annex I listing of the active ingredient, it was already mentioned that surfactants could significantly contribute to the toxicity of glyphosate products.

To facilitate a comprehensive risk assessment of products that contain both glyphosate and the POE-[REDACTED] and to ensure sufficient protection of operators, bystanders, workers, residents and consumers, it was necessary to establish reference values (ADI, AOEL, ARfD) for the surfactant with CAS no. 61791-26-2 and to estimate its dermal absorption rate. These values may be applied in future for risk assessment purposes in addition to those of glyphosate.

Another goal of this evaluation was to check if there was enough evidence to conclude that higher toxicity of certain PPP was in fact due to the surfactant or if there were indications of a synergistic mode of action with glyphosate.

Conclusion:

A systemic AOEL, an ADI and an ARfD in the same magnitude of 0.1 mg/kg bw/(day) are proposed for the POE-[REDACTED] with the CAS no. 61791-26-2 that is contained as a surfactant in many glyphosate-based (and some other) PPP. Furthermore, an inhalative AOEL of 0.0166 mg/kg bw/day was established.

For its dermal absorption rate, in the absence of experimental data, the default values of 25% or 75%, depending on concentration, are proposed.

The substance should be classified and labelled for acute oral toxicity (Xn, R22, corresponding to “Acute tox. 4, H302” according to GHS), for skin and severe eye irritation (Xi, R38-41; corresponding C&L according to the GHS would be “Skin irrit. 2, H318” and “Eye dam. 1, H318) and skin sensitisation (Xi, R43, corresponding to “Skin sens. 1, H302”). Most likely, classification for inhalative toxicity will be also needed.

With regard to nearly all toxicological endpoints under investigation, the POE- [REDACTED] was clearly more toxic than glyphosate. Its primary mode of action was a local effect, i.e., strong mucosal irritation. However, occurrence of systemic effects after ingestion or inhalation is also likely. There is some evidence to assume a higher vulnerability of pups.

Eye or mucosal irritation may be produced by both glyphosate and the surfactant and some additivity seems theoretically possible but, with regard to the very different toxic properties of these substances and the apparent differences in the effect doses and severity of findings, higher toxicity of certain PPP as compared to the active ingredient can be allocated to the surfactant alone. The same holds true for poisoning incidents in humans. Therefore, separate reference values of these products are not needed but risk assessment should include a comparison of the expected exposures to the reference values for both glyphosate and [REDACTED] surfactant.

Justification (detailed evaluation):

The evaluation of and proposed reference values for the POE- [REDACTED] surfactant with the CAS no. 61791-26-2 are mainly based on toxicological studies with the surfactant formulations MON 0818 and G-3780 that were submitted by the company Monsanto on request of the BfR after they had been identified in a recent evaluation of the U.S. Environmental Protection Agency (EPA). Both formulations contain this [REDACTED] at an amount of about 70 % and are or were part of various glyphosate-based PPP. The data package consists of subchronic oral studies in rats and dogs and of reproductive and developmental toxicity studies in rats. Furthermore, former evaluations by the EU, the EPA and a number of publications were taken into consideration. With regard to the EPA evaluation (EPA, 2009, ASB2009-9022), it must be emphasised that it was more comprehensive because the whole group of polyethoxylated alkyl amines was addressed with special regard to the need of setting tolerances in crops. The EPA conclusions were drawn under the assumption that the alkyl amine content in herbicidal formulations will not exceed 25 % and will not be higher than 10 % in fungicides and insecticides.

In the following, an overview on the toxicological profile of the POE- [REDACTED] surfactant with CAS no. 61791-26-2 is given. (For direct comparison to glyphosate, see Table B.6.13-2.) Subsequently, reference values are proposed, the dermal absorption rate is estimated and the possible impact of the surfactant on the toxicity of Roundup® formulations as an example for glyphosate-based herbicides is discussed.

Toxicokinetics and metabolism

No information is available.

Acute toxicity

The acute oral LD₅₀ of the surfactant in rats was 864 mg/kg bw when the value of ca 1200 mg/kg bw for MON 0818 (EPA, 2009, ASB2009-9022) was corrected for the presumed POE- [REDACTED] content of 72 %. This result warrants classification and labelling

for acute oral toxicity as “harmful if swallowed” (Xn), *i.e.*, the risk phrase R22, or H302 (Acute toxicity cat. 4, “Warning”) according to GHS would be appropriate.

The acute dermal toxicity was tested in rabbits. The LD₅₀ was above the highest dose of 907 mg/kg bw when corrected for [REDACTED] content. Although the amount applied was below the required limit dose of 2000 mg/kg bw, classification and labelling is not considered necessary because no mortality occurred and no clinical signs were reported up to this dose.

Unfortunately, acute inhalation data for the POE-[REDACTED] under consideration or a surfactant formulation such as MON 0818 is not available. This must be in fact regarded as a data gap because there is evidence coming from an acute inhalation study with a Roundup formulation (Velasquez, 1982, TOX2002-693) that inhalative toxicity was higher than with glyphosate alone for which an LC₅₀ > 5 mg/L air was determined (see Volume 3, B.6.2.3 of this RAR). In this acute study, an LC₅₀ of 3.18 mg/L air for Roundup was obtained resulting in the classification and labelling of the product with Xn, R20 (H332 according to GHS). It is quite likely that this apparent difference was due to the surfactant. MON 0818 was contained in this product at an amount of approximately 18 % w/v, *i.e.*, the POE-[REDACTED] content was *ca* 14 % w/v. When the strong irritating properties of the POE-[REDACTED] surfactant (see below) are taken into consideration, one would expect similar effects in the respiratory tract. A higher inhalative toxicity than for glyphosate was also substantiated by a subacute inhalation study ([REDACTED] 1983, TOX2002-694) with Roundup and by observations in humans after occupational exposure without protective measures ranging from weak symptoms such as a headache to well-documented systemic poisoning with persistent morphological findings ([REDACTED] 1991, MET9600092; [REDACTED] 2007, ASB2013-4034).

A high acute inhalative toxicity was experimentally confirmed for other polyethoxylated alkyl amine substances. *Armoblen 557* (CAS no. 68219-26-3) had an LC₅₀ of 0.66 mg/L that was established in a study with 4-hour exposure. For *Ethomeen C/12* (CAS No. 61791-31-9), an LC₅₀ of 0.98 mg/L was calculated on the basis of a one-hour trial (EPA, 2009, ASB2009-9022).

However, acute inhalation data for different products that were submitted to support registration in Germany were partly contradictory although they contain similar amounts of glyphosate (at least 360 g/L) and of the surfactant. Apparently, there is no clear correlation of inhalative toxicity with the surfactant content. In fact, there are PPP with the same surfactant at nearly the same concentration for which classification and labelling for acute inhalation toxicity is not needed. In sum, the available information is not sufficient to conclude on the appropriate classification and labelling of the POE-[REDACTED] surfactant itself for acute inhalation toxicity although some classification will be probably needed.

If experimental data for a particular formulation containing this surfactant is not available, according to Directive 1999/45/EEC or the CLP regulation, classification and labelling of the PPP for precautionary reasons might be a reasonable option.

The POE-[REDACTED] surfactant was found irritating to the skin (Xi, R38) and strongly irritating to the eyes. The U.S. EPA (EPA, 2009, ASB2009-9022) labelled the substance for eye effects even as “corrosive” (C) but, according to the EU scheme, R41 seems to be more appropriate. Correct classification according to GHS rules difficult because the studies themselves were not submitted and the assessment is based on the EPA evaluation. However, in Annex VII of the CLP regulation (Translation table from classification under Directive 67/548/EEC to classification and assignment of hazard statements under this regulation) is

applied, Cat.2/H315 for skin irritation and Cat.1/H318 (for eye irritation) would be most appropriate.

Eye irritation is often considered to provide evidence also of mucosal irritation. (1987, TOX9552430) studied the irritating effect of the glyphosate isopropylammonium salt, MON 0818 and a Roundup formulation (containing 41% w/v of the IPA salt and 15% of MON 0818) on stomach and small intestine mucosa in dogs. Irritation was more severe with the Roundup formulation than with either the IPA salt or the surfactant alone. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused by 0.25 N hydrochloric acid.

Concerning skin sensitisation, the available data is scarce. However, in its 2009 evaluation, U.S. EPA concluded that the POE- was sensitising because of a positive Buehler test using three applications (EPA, 2009, ASB2009-9022). Therefore, classification and labelling (Xi, R43 or, according to GHS, Skin sensitisation, Cat.1/H317) is proposed.

For setting of reference values, the acute toxicity data is, of course, not appropriate. However, acute oral toxicity supports a need to establish an ARfD and the evidence of inhalative toxicity suggests that an inhalative AOEL should be established.

Short-term toxicity (subacute and subchronic studies)

Rat, oral

A 90-day feeding study was performed on SD rats (1990, ASB2009-9027). Because current standards were basically met, the study may be considered as valid and reliable. Test substance was the surfactant formulation MON 0818. As stated in the study report, this test item contained 71.9 % of “polyoxyethylen (15) tallow amine” (POEA). Groups of 10 male and 10 female rats were fed MON 0818 at nominal concentrations of 0, 500, 1500, or 4500 ppm. In most groups, however, these nominal concentrations were not achieved. The actual concentrations were in the range from 80 to 90 % of the nominal values.

The highest dose level was clearly toxic. This became apparent mainly by a reduced body weight gain in both sexes that achieved statistical significance and resulted in a lower mean body weight over the whole course of the study. Food consumption was diminished and clinical signs (piloerection, soft faeces) occurred. Furthermore, blood glucose and urea levels were decreased.

Some deviations in organ weights are most probably related to the lower body weight at termination and there were no gross pathological findings at necropsy that could be attributed to treatment. In contrast, histological lesions of the intestinal mucosa were certainly treatment-related. These findings comprised hyperplasia and cell vacuolation in the *Lamina propria* and all animals receiving the high dose were affected.

At the mid dose level of 1500 ppm, 4 out of ten male rats and 5/10 females exhibited the same histopathological changes as described above. In males, there was in addition a statistically significant decrease in food consumption and body weight gain over the first 9 days of treatment.

Feeding of MON 0818 at the low dose level of 500 ppm did not cause any remarkable differences from the control groups and, thus, this dose can be regarded as the NOAEL.

For the nominal dietary concentration of 500 ppm, a mean daily intake of 33.0 mg/kg bw was calculated for the male rats and of 39.9 mg/kg bw for the females. However, to establish the true NOAEL for the POE- these values must be corrected for the actually

achieved concentration of 84% of nominal in both the male and female groups and then for the 71.9 % content of the surfactant in MON 0818. These corrections result in intake calculations of 19.9 mg/kg bw/day for males and 24.1 mg/kg bw/day for females. It seems reasonable to round them to 20 mg/kg bw/day that is considered the NOAEL for the surfactant in this study. For the LOAEL that was established at a three times higher dietary level, hence, an achieved intake of 60 mg/kg bw/day is assumed.

The outcome of this study 90-day was at least partly in line with an additional one-month feeding study with "POEA surfactant" on Sprague-Dawley rats of which the original report was not made available to the BfR. In this experiment, the NOAEL was claimed to be 800 ppm (*ca* 40 mg/kg bw/day). At the next higher dose level of 2000 ppm, body weight gain in male rats was reduced. At the high dose level of 5000 ppm, lower body weight gain and irritation and inflammation of the colon mucosa were observed in both sexes [REDACTED], 1989, cited in [REDACTED], 2000, ASB2012-12053).

The toxicological findings in these feeding studies in rats point to local irritation in the intestines as the primary mode of action. Because of the known irritating properties of the POE- [REDACTED] it was first assumed that this local effect was behind the higher toxicity of PPP containing glyphosate and this surfactant as compared to the active ingredient. However, the subchronic study in dogs, the reproduction and developmental studies in rats, comparative mechanistic data and human experience suggest that systemic effects will be most likely due to a second mechanism of POE- [REDACTED] toxicity.

Dog, oral

Groups of four male and four female Beagle dogs received three times a day a capsule containing the formulation G-3780 for a total of 14 weeks ([REDACTED] 1973, ASB2009-9026). According to a claim made by Monsanto, G-3780 was very similar to the formulation MON 0818. However, in the study report itself, no information on the composition of the test substance and the content of the POE- [REDACTED] with the CAS no. 61791-26-2 is given. Therefore, it is assumed that the POE-content, as in MON 0818, was about 72% suggesting a need for correction of the NOAEL/LOAEL.

The total daily doses of G-3780 were 0, 30 (*i.e.*, 3 x 10), 60 (3 x 20), or 90 (3 x 30) mg/kg bw. However, because of a rather tricky dosing scheme, these dose levels were achieved not before the third or fourth week of treatment. This is one of the reasons for a compromised reliability of this study. Transiently, high dose animals even received 120 (3 x 40) mg/kg bw/day but, because of enhanced emesis, diarrhoea and subsequent emaciation, the dose was lowered again after 10 days. It seems that the MTD was exceeded when more than 90 mg/kg bw/day was administered.

At the two upper dose levels, dogs did not gain and sometimes even lost weight. At termination, mean body weight were by more than 10% lower than in the control groups. In addition, vomiting and diarrhoea were more frequently observed than in the controls. Clinical chemistry revealed lower blood calcium and total protein concentrations.

There were no remarkable findings at necropsy and histopathological examination. In particular, local effects on intestinal mucosa, as in the rat study, were not reported. The clinical signs might suggest gastrointestinal irritation but, on the other hand, are common and unspecific signs of general toxicity in dogs.

The lowest dose level of 30 mg/kg bw/day was considered the NOAEL. After correction for presumed POE- [REDACTED] content, a numeric value of 21 mg/kg bw/day would result that is in the same magnitude as the NOAEL in the subchronic rat study. The LOAEL was 42 mg/kg bw/day.

Despite a wide range of parameters that were investigated, the study design, examination methods and the quality of reporting do not comply with modern standards. Therefore, and because of the uncertainties with regard to the actually applied doses, the study can be considered at best supplementary. However, it can be accepted that the requirement of testing for short-term toxicity in a second species has been fulfilled. The conclusion can be drawn that the sensitivity of rats and dogs in terms of the NOAELs/LOAELs is not that different. In contrast to the studies in rats, however, the findings in dogs suggest rather a systemic effect than local irritation.

Rat, inhalative

In a four-week study on SD rats, 15 male and 15 female animals per group were exposed (whole-body) for 6 hours per day over 5 days per week (total number of treatments 22) to nominal concentrations of 0 (control), 0.37, 0.75, or 2.17 mg/L air of the Roundup formulation MON 2139 (1983, TOX2002-694). However, the analytically determined air concentrations were 0.05 mg/L (low dose), 0.16 mg/L (mid dose), and 0.36 mg/L (high dose). There were no unscheduled deaths in this study. Clinical signs did not occur, and there were no gross pathological changes at necropsy. Body weight and organ weights were not altered but, surprisingly, the lungs had not been weighed.

The only findings that may be attributed to treatment were significantly higher total protein, albumin and globulin serum concentrations in females at the two upper dose levels. Furthermore, the incidence of certain histopathological findings in the lungs (perivascular lymphoid infiltrates or aggregates, interstitial infiltration or pneumonia), in the trachea (mononuclear infiltration and chronic inflammation) and the nasal turbinates (inflammation) was increased in high dose females. Unfortunately, histopathology was performed on control and high dose animals only.

Even though the study author did not mention these findings as adverse or treatment-related, they might be a reaction to Roundup application. Accordingly, the lowest concentration of 0.05 mg/L air is considered the NOAEC.

Under the assumption that the effects were in fact entirely due to the POE- an inhalative NOAEL (expressed in mg/kg bw) for this surfactant may be calculated. Since 180 g/L MON 0818 were contained in the test item, a NOAEC of 0.009 mg/L air for this surfactant formulation can be assumed. Taking into consideration the content in MON 0818, a respiratory rate of 45 L air/kg bw per hour for the rat and an exposure time of 6 hours per day, the calculation would result in a inhalative NOAEL of ca 1.66 mg/kg bw/day.

Mutagenicity

Possible mutagenicity of POE- (s) was addressed in the past by different regulatory bodies as well as in a review article (Germany, 2000, ASB2013-2748; 2000, ASB2012-12053; EPA, 2009, ASB2009-9022). The overall conclusion was that these substances were not mutagenic but might have caused positive findings in a number of test systems due to cytotoxicity when PPP such as various Roundup formulations were tested. Unfortunately, there are relatively few experiments with formulations in standard test systems available because usually only the active compounds are subject to rigorous testing in a battery of regulatory studies. At least, the Roundup formulation MON 2139 containing the POE- with the CAS no. 61791-26-2 as part of the surfactant formulation MON 0818 proved negative in an Ames test in concentrations of up to 500 µg/plate without and of up to 1500 µg/plate in the presence of metabolic activation by S9 mix (1992, TOX1999-239). In a mouse bone marrow micronucleus assay (1992, TOX1999-242), no evidence of a clastogenic potential was found up to the highest tested dose of 555

mg/kg bw/day that was already clearly toxic to mice after single intraperitoneal administration. Roundup proved cytotoxic in the bone marrow.

MON 0818 itself was tested in the Ames test by Stegemann and Li (1990, TOX1999-241) and proved negative. However, due to severe cytotoxicity, it could be tested only at rather low concentrations (up to 300 µg/plate) in the various *Salmonella typhimurium* strains. It proved also negative in a micronucleus assay in mouse bone marrow (Stegemann and Kier, 1998, TOX1999-240) at the dose level of 100 mg/kg bw that was administered by intraperitoneal injection but this latter study was considered supplementary only because no evidence of systemic or bone marrow toxicity was obtained.

There is evidence coming from several studies using the Comet assay or other less validated systems, that products which contain cytotoxic [REDACTED] (or other) surfactants might produce, mostly in very high concentrations, DNA damage either by direct contact or by enhanced formation of DNA reactive oxygen species (e.g., [REDACTED], 1997, Z101728; [REDACTED] 1998, TOX1999-318; [REDACTED] 2008, ASB2012-11586). A part of these investigations was made in non-mammalian systems that are more relevant to ecotoxicology. Positive findings are nearly always linked to toxicity as recently confirmed by Heydens *et al.* (2008, ASB2012-11845). Unfortunately, no UDS assay in, e.g., rat hepatocytes is available that would be most suitable to investigate a potential for DNA damage also at concentrations below overt toxicity. However, for the time being, weight of evidence suggests that the relevance of possible effects on the DNA to humans under practical exposure conditions is very low.

Chronic toxicity and carcinogenicity

No such data is available. Currently, for co-formulants like surfactants, long-term studies are not required and, accordingly, usually not performed. However, based on the toxicological profile of POE-[REDACTED] it is not expected that chronic toxicity would be much different from the effects that were noted in the subchronic studies. With regard to carcinogenicity, it should be taken into consideration that, in spite of long-lasting experience and extensive use, there is no convincing epidemiological evidence in people who had been in frequent occupational contact with glyphosate-based plant protection products (see Volume 3, B.6.5, and Volume 1, 2.6.5 of this RAR). The EPA concluded that the whole group of polyethoxylated alkyl amines was not of concern for carcinogenicity (EPA, 2009, ASB2009-9022).

Reproduction toxicity

Two GLP-compliant studies were performed in rats to investigate possible effects of the surfactant formulation MON 0818 on reproduction [REDACTED], 2007, ASB2010-365; [REDACTED] 2008, ASB2010-364). Furthermore, a rather new published study on reproductive toxicity of a commercial Roundup® formulation ([REDACTED] 2007, ASB2012-2721) is available. Unfortunately, the design of all three studies was not in line with usual OECD Guideline requirements. The reproductive/developmental screening studies according OECD testing guidelines 421/422 are less sensitive than the full scale study designs according to testing guidelines 414/416.

MON 0818

In a two-generation study (according to OECD 421), Sprague-Dawley rats (20 per sex and dose group) received the test formulation in the F0 generation at dose levels of 0, 100, 300 or 1000 ppm via their diet (■■■■■ 2007, ASB2010-365). A broad range of endpoints was examined including hormone measurements, sperm parameters and extensive histopathology. There was no evidence of parental toxicity but the highest dose tested was lower than the LOAEL in the 90-day rat study. In contrast, administration of the high dose resulted in a reduced implantation rate (with 5 of 15 pregnant dams being affected), lower litter size and also an increase in perinatal mortality. Total loss of two litters immediately after birth or at the beginning of the lactation period was noted. However, further development of the surviving pups was not altered.

To produce the F2 generation, three male and female pups from each litter were selected and reared. Between days 21 and 70 after birth, they received the test item at a dietary concentration that was adjusted to the respective actual body weight. The mean daily doses during this period were 0, 7, 21 or 61 mg/kg bw in male rats and 0, 6, 28 or 72 mg/kg bw in females. Two male and female pups per litter were selected for further breeding but only from the control and high dose groups. From the beginning of this third mating period, the selected high dose F1 animals received again 1000 ppm of MON 0818 in their diet. In the F2 generation, no parental toxicity and no impact of this high dose on reproduction were observed.

Histopathological examination of different parts of the intestinal tract (jejunum, ileum, caecum, colon and rectum) did not reveal indications for mucosal irritation in F0 and F1 animals.

Based on the findings in the F1 generation (i.e., lower implantation rate, litter size and pup survival) 300 ppm was considered the NOAEL for reproduction and offspring effects. For the F0 adults, a mean daily intake of 16.6 (males) or 19.5 mg/kg bw/day of MON 0818 was calculated at this dose level that must be corrected for the ■■■■■ surfactant content. A NOAEL of 12 (males) to 14 (females) mg/kg bw/day for the surfactant would result (EPA, 2009). For parental toxicity, the highest dose of 1000 ppm was the NOAEL. The corrected mean daily surfactant intake was about 38 mg/kg bw (lowest value as calculated for F1 males).

In a second study (■■■■■ 2008, ASB2010-364); according to OECD 422), groups of 12 male and female rats of the same strain [CrI:CD(SD)] were fed 1000 ppm of MON 0818 for a total period of 69 – 72 days. This dose was equivalent to a mean daily intake of 66 mg/kg bw by the male animals and 95 mg/kg bw/day by females over the whole course of the study. During the individual study periods (pre-mating, pregnancy, lactation), the intake in females varied between 74 and 126 mg/kg bw/day. A control groups of equal size received untreated diet. From administration day 14 onwards, the animals were mated.

There were two unscheduled deaths among the treated dams. One female rat died showing clinical signs of dystocia. Another dam was did not give birth and was killed 30 days after mating in poor clinical state. At necropsy, uterine rupture was established and two implantation sites were recorded of which one was a dead fetus and the other an early resorption. In contrast to the assessment by the study author, it cannot be excluded that these deaths were treatment-related. However, there were no adverse effects in the remaining 10 dams or in any of the 12 males in the treatment group. Furthermore, reproduction was not affected in the remaining dams. A higher incidence of chronic-progressive nephropathy in treated males as compared to the control group (6/12 vs. 3/12) was not allocated to substance administration because it was only unilateral. Thus, a NOAEL for parental and reproduction

toxicity was not established. Offspring effects could not be fully evaluated because all pups were killed on day 4 *post partum* already. However, if effects on litter size or perinatal mortality as in the first study would have occurred, they might have been noted.

Roundup® herbicide

In a one-generation study, pregnant Wistar rats (15 per group) were administered a Roundup® herbicide that is commercially available in Brazil (containing 36% glyphosate and 18% of the POE- [REDACTED] surfactant) at dose levels of 50, 150, or 450 mg/kg bw/day by oral gavage from day 1 of presumed gestation through the end of lactation (postnatal day 21). The calculated daily intake of the surfactant was 9, 27, or 81 mg/kg bw. A control group received only the vehicle, *i.e.*, distilled water ([REDACTED] 2007, ASB2012-2721).

Evaluation of toxicity in the parental generation was based on observations for mortality and clinical signs, body weight measurements and determination of selected organ weights at termination. Reproductive toxicity was assessed by determining litter size, number of living and dead pups, viability and sex ratio. Possible effects on offspring development, in particular with regard to sexual maturation, were studied in one male pup and one female pup per litter which were killed at an age of 65 days and in one more pup per sex and litter which were sacrificed on postnatal day 140. Organ weights were also determined in these animals.

Up to the highest dose level, there was no evidence of maternal toxicity. Likewise, litter size, mean number of live and dead pups and sex ratio among the pups were not affected at any dose level. Thus, the NOAEL for parental and reproductive toxicity was 450 mg Roundup/kg bw/day, corresponding to a dose of 81 mg/kg bw/day for the surfactant.

However, sexual development in offspring was affected. In female pups, delayed vaginal opening suggested a slower sexual development in all treated groups. Since this finding was associated with a markedly lower body weight in the low dose group, a possible treatment-related effect was assumed only for the two upper dose levels. In male pups, functional disturbances were confined to the highest dose level of 450 mg Roundup/kg bw/day and were partly contradictory. On one hand, preputial separation was noted to occur a bit earlier than in the control group. On the other hand, at an age of 65 days, testosterone serum concentration was significantly lower (but not at 140 days) and, when measured at 140 days, daily sperm production and total number of sperm in the epididymis tail were diminished suggesting rather a delay in sexual development. Interpretation of this data is difficult since a similar decrease in both parameters was observed at the low dose level, too, whereas the mean values in the mid dose group were similar to the controls. At all three dose levels, histopathological examination revealed degenerative changes in the testes with the absence of tubular lumen being the most outstanding finding. However, all these observations were flawed by a low number of animals on which the findings are based, *e.g.*, histopathology of the testes was confined to only five male pups per dose level.

Thus, a NOAEL for offspring effects cannot be established.

A direct comparison of the results by [REDACTED] (2007, ASB2012-2721) with the two studies of [REDACTED] (2007, ASB2010-365 and 2008, ASB2010-364) is not possible because the test material was different and the same holds true for the study design and the range of parameters under investigation. Unfortunately, it is also not feasible to compare these studies and their results with the Guideline-compliant reproduction studies with glyphosate. Thus, no definite conclusion can be drawn if the effects of treatment with Roundup can be allocated to the surfactant. Nonetheless, the published findings suggest that offspring development was in fact a particularly sensitive target of Roundup and the POE- [REDACTED]. The findings in young male rats might indicate impairment of spermatogenesis.

Developmental toxicity

The surfactant formulation MON 0818 and a Roundup® herbicide that is commercially available in Brazil were tested for developmental toxicity and teratogenicity in rats. A study in a second species was not submitted. However, it is unclear whether the data requirements for pesticides or drugs can be applied to co-formulants. Furthermore, the rabbit as the usual second species is known to be very sensitive to gut irritation. Thus, severe maternal toxicity at low doses due to the well known irritation potential of the [REDACTED] surfactant must be expected in a rabbit study that might prevent meaningful evaluation of fetal effects at sufficiently high dose levels.

MON 0818

A preliminary (range-finding) and a main study were performed under GLP conditions in which MON 0818 was administered to pregnant rats (Charles River Crl:CD Br). Although the studies themselves can be considered acceptable, it is not clear how much POE- [REDACTED] was contained because its amount even in different batches of MON 0818 can vary. However, based on the information given in the study report of [REDACTED] (1990, ASB2009-9027) and in line with the EPA evaluation (EPA, 2009, ASB2009-9022), it is assumed that the amount of this surfactant in the tested formulation had accounted for 71.9 %, too. The NOAELs/LOAELs will be corrected accordingly.

Preliminary study

MON 0818 was administered by oral gavage to groups of 8 pregnant rats from day 6 through day 15 post mating at dose levels of 0, 25, 50, 100, 200, or 400 mg/kg bw/day. On pregnancy day 20, the dams were killed and fetuses developed by caesarian section. The uteri were dissected and examined. Foetuses were counted and inspected for external anomalies.

Mortality, clinical signs and body weight losses were clear indications of severe maternal toxicity at a dose of 100 mg/kg bw/day and above. The NOAEL for maternal toxicity was 50 mg MON 0818/kg bw/day, corresponding to 36 mg/kg bw/day for the POE- [REDACTED]. Obvious developmental toxicity, in contrast, was confined to the highest dose level of 400 mg/kg bw/day at which post implantation losses were increased. However, due to mortality among the dams, only 3 litters were available at this dose for evaluation preventing meaningful evaluation of teratogenicity ([REDACTED] 1989, ASB2009-9028). Based on these results, the dose selection for the subsequent main study appears acceptable.

Main study

25 pregnant rats per groups received MON 0818 from days 6 through 15 post mating by oral gavage at dose levels of 0, 15, 100 and 300 mg/kg bw/day. Following sacrifice of the dams and caesarean section, uteri were inspected and fetuses examined for external, visceral and skeletal anomalies by appropriate methods ([REDACTED] 1990, ASB2009-9029).

Severe maternal toxicity became apparent at the top dose level by the death of 6 dams between treatment days 8 and 13, clinical signs, initial body weight losses and a diminished body weight gain thereafter. Furthermore, food consumption was decreased. After cessation of treatment, body weight gain and food consumption showed a trend towards normalisation from study day 16 onwards. Soft and mucous faeces might suggest mucosal damage. A lower mean liver weight was probably a reflection of the lower body weight.

At the mid dose level of 100 mg/kg bw/day, mean food consumption was significantly reduced during the first three days of treatment and five out of 25 dams lost weight although the mean body weight and body weight gain were not different from the control group. Clinical signs were only rarely seen. Based on these minor findings, and in accordance to the study author, this dose is considered the LOAEL. For this assessment, it was also taken into

account that clear maternal toxicity occurred at the same dose level in the range-finding study. After adjustment for a [REDACTED] content of 71.9 %, the LOAEL for the surfactant was calculated to be 72 mg/kg bw/day. The low dose of 15 mg MON 0818/kg bw/day was the NOAEL in this study corresponding to 10.8 mg [REDACTED]/kg bw/day. (In the 2009 EPA evaluation (ASB2009-9022), the findings at 100 mg MON 0818/kg bw/day were disregarded and, accordingly, 72 mg/kg bw/day was considered the NOAEL for the [REDACTED]. This dose was also used as starting point to derive the ARfD.)

In the study report ([REDACTED] 1990, ASB2009-9029) as well as in the EPA evaluation (EPA, 2009, ASB2009-9022), it is stated that no developmental toxicity was observed up to the highest dose level of 300 mg/kg bw/day. However, the total number of visceral and skeletal anomalies at this dose was increased. Due to maternal mortality, only 15 litters were available for evaluation but the incidence of exencephalia and stenosis of the right carotid were already above the historical control range. It may be expected that, with a higher number of litters, the frequency of these anomalies would be even higher. Furthermore, malformations such as *Situs inversus* and absent bladder were noted only in this high dose group for which no historical control data was provided. Therefore, the NOAEL for developmental toxicity of MON 0818 was the mid dose level of 100 mg/kg bw/day corresponding to 72 mg/kg bw/day for the [REDACTED]. The highest dose of 300 mg/kg bw/day (216 mg/kg bw/day) was considered the LOAEL for this endpoint.

Roundup® herbicide

Pregnant Wistar rats (14 - 16 per group) were administered a Roundup® formulation that is commercially distributed in Brazil and was reported to contain 36% glyphosate and 18% of the POE- [REDACTED] surfactant [REDACTED] 2003, ASB2012-11600). The test material was applied in distilled water once a day by oral gavage from day 6 through 15 of gestation at dose levels of 500, 750, or 1000 mg/kg bw whereas the control group received only the vehicle. The respective doses of the [REDACTED] accounted for approximately 91, 135, and 180 mg/kg bw/day. On gestation day 21, dams were anaesthetised and the uteri with contents were removed by caesarean section and weighed. Afterwards, the dams were sacrificed, necropsied and organ weights of heart, lung, liver, kidneys, and spleen determined. Uteri were inspected for live and dead fetuses and number of implantation sites. The fetuses were weighed, sexed and examined for external and skeletal but not for visceral anomalies.

Maternal toxicity was severe with 50% of the dams (7 out of 14 in that group) dying between gestation days 7 and 14 but was confined to the highest dose level of 1000 mg Roundup/kg bw/day. In the surviving dams, no remarkable findings were reported and relative organ weights did not show statistically significant differences although relative liver weight at the top dose level tended to be increased.

Developmental toxicity was observed in all dose groups and was characterised by a developmental delay of the skeleton and an increase in certain skeletal anomalies. Whereas only 15.4% of the fetuses in the control group exhibited skeletal findings of any kind, the total frequency was higher in a dose-related manner in the treated groups (33.1%, 42.0%, and 57.3%). Incomplete ossification of the skull was noted in all three dose groups and was dose-related as well as a reduced number of caudal vertebrae at the two upper dose levels. In contrast, for other findings, a clear relation to dose was absent. Thus, the incidence of the malformation "fused zygomatic bone" was higher only at the lowest dose level and, accordingly, cannot be attributed to treatment.

The NOAEL for maternal toxicity of the POE- [REDACTED] in a Roundup formulation in this study was 135 mg/kg bw/day. In contrast, a NOAEL for fetotoxicity/teratogenicity could not be established.

Similar maternal or developmental effects in rats were not reported for the active ingredient glyphosate (see Volume 3, B.6.6 and Volume, 2.6.6 of this RAR). It may be concluded that the higher maternal and developmental toxicity of Roundup® was due to co-formulants in the herbicidal formulation. It is quite likely that they result from the rather high amount of the POE- [REDACTED] surfactant in the product but a definitive proof is lacking. It must be emphasised that the selected dose levels in the more recent Brazilian study were clearly above the LOAELs in the 90-day study ([REDACTED] 1990, ASB2009-9027) and the developmental studies ([REDACTED] 1989, ASB2009-9028; [REDACTED] 1990, ASB2009-9029) with the surfactant in the formulation MON 0818. However, these previous studies were apparently not known to the authors because the reports were unpublished. When the different studies are compared, it is surprising that maternal toxicity in the study by [REDACTED] (2003, ASB2012-11600) occurred only at the highest dose level. It may be doubted if the investigations in the dams were sufficiently extensive to reveal adverse findings at the lower dose levels. In contrast, developmental (skeletal) effects were obviously more pronounced as in the more comprehensive study by [REDACTED] (1990, ASB2009-9029).

Neurotoxicity

No data available. Based on chemical structure, a specific neurotoxic potential is not expected. The available studies do not suggest neurotoxicity of the [REDACTED] surfactant.

Mechanistic studies

A systemic effect of a [REDACTED] surfactant was demonstrated by [REDACTED] (1990, TOX9552419) who studied the haemodynamic effects of continuous i.v. application of either glyphosate IPA salt, the formulation Roundup or the surfactant in dogs. The impact on cardiovascular functions was studied in groups of five anaesthetised and artificially ventilated female Beagle dogs. Duration of i.v. exposure was 60 minutes. A total of 8.2 g glyphosate (IPA salt administration) or 2.8 g glyphosate (Roundup) was injected. These amounts would correspond to doses of about 550 - 820 mg/kg bw or 180 - 280 mg/kg bw, respectively, since the body weight of the dogs was 10 to 15 kg.

The surfactant alone and Roundup significantly reduced the blood pressure, cardiac output and left ventricular stroke work index suggesting a marked effect on circulation. It could be shown that the cardiac depression observed with Roundup was likely due to the surfactant since, in contrast, arterial blood pressure was even increased when glyphosate isopropylamine salt had been injected. Similarly, the IPA salt did not cause changes in heart rate or cardiac output. A decrease in blood pH observed in this group could be either due to a direct effect of administration or to metabolic acidosis. In any case, it was not strong enough to affect the circulatory system.

[REDACTED] (1990, Z44833) reported a high toxicity of a [REDACTED] following intratracheal application to dogs, due to severe lung irritation. A similar effect after oral administration was assumed to result from aspiration because vomiting occurred.

Human data (poisoning incidents)

Despite the low acute toxicity of glyphosate technical, a number of poisoning incidents in humans sometimes even resulting in death were reported in particular from Asian countries (DAR, 1998, ASB2010-10302). Severe intoxication was mainly characterized by a decrease

in blood pressure and further cardiovascular symptoms followed by pulmonary dysfunction and renal failure and by signs of irritation in the gastrointestinal tract. Pathophysiology of poisoning by the oral route is assumed to include irritation or corrosion of the intestinal mucosa as a first step resulting in electrolyte imbalances, shock and disturbances in the cardiovascular system. The respiratory signs, as well as renal symptoms, are considered secondary to this mechanism being caused either by pulmonary edema related to disturbed circulation or by aspiration pneumonia following emesis. It is generally assumed that all these effects were mainly due to the () surfactants () 2004, ASB2012-11576; () 2004, ASB2012-12038), as well as disturbances of lung function and circulation and histopathological lung lesions after acute inhalation () 2007, ASB2013-4034). () (1987, Z35531) reported two cases of human poisonings with surfactants causing clinical signs resembling very much those observed after ingestion of large amounts of Roundup.

Reference doses

Table B.6.13-1 provides an overview on the toxicological studies with the surfactant formulations MON 0818 or G-3780 that might be used for setting reference values for the POE- () with the CAS no. 61791-26-2. For correction of the NOAELs/LOAELs, a () content of 72% is assumed. Although acute studies might point to, e.g., a need for setting an ARfD, they are usually not considered an appropriate basis to derive any of the reference doses. Likewise, mechanistic studies are often performed under very artificial conditions and unrealistic high doses are employed. Long-term studies that are mostly used to derive the ADI are not available. Therefore, only short-term toxicity, reproduction and developmental toxicity studies can be taken into consideration for this purpose.

Table B.6.13-1: NOAELs and effect doses for the POE- [REDACTED] with CAS no. 61791-26-2 in relevant toxicological studies

Study type / Formulation	NOAEL	LOAEL	Effects at LOAEL	Reference
90-days (feeding), rat / MON 0818	20 mg/kg bw/d	60 mg/kg bw/d	Histopathological lesions of intestinal mucosa, bw gain and food consumption ↓	[REDACTED] 1990 ASB2009-9027
14-weeks (capsules), dog / G-3780	21 mg/kg bw/d	42 mg/kg bw/d	Clinical signs (vomiting, diarrhea); bw (gain) ↓	[REDACTED] 1973 ASB2009-9026
4-weeks (inhalation), rat / MON 2139 (Roundup)	1.6 mg/kg bw/d (calculated from NOAEC)	5 mg/kg bw/d (calculated from LOAEC)	Clinical chemistry findings, histopathological lesions (lung, trachea, nasal turbinates) at higher concentration	[REDACTED] 1983 TOX2002-694
2-generation (feeding), rat / MON 0818	12 mg/kg bw/d	38 mg/kg bw/d	Implantation rate, litter size, pup survival ↓	[REDACTED] 2007 ASB2010-365
One-generation (feeding), rat / MON 0818	Not established	74 mg/kg bw/d	Equivocal evidence of maternal toxicity	[REDACTED] 2008 ASB2010-364
One-generation (feeding), rat / Roundup	81 mg/kg bw/d (reproductive and parental toxicity), not established for offspring effects	> 81 mg/kg bw/d (reproduction and parental toxicity); 9 mg/kg bw/d (offspring effects)	Sexual development mainly of male pups affected	[REDACTED] 2007 ASB2012-2721
Developmental toxicity (gavage), rat (range-finding study) / MON 0818	36 mg/kg bw/d	72 mg/kg bw/d	Clinical signs and bw losses in pregnant dams	[REDACTED] 1989 ASB2009-9028
Developmental toxicity (gavage), rat (main study) / MON 0818	10.8 mg/kg bw/d (maternal toxicity); 72 mg/kg bw/d (developmental effects)	72 mg/kg bw/d (maternal toxicity); 216 mg/kg bw/d (developmental effects)	Food consumption ↓ and bw losses in dams; visceral and skeletal anomalies in fetuses ↑	[REDACTED] 1990 ASB2009-9029
Developmental toxicity (gavage), rat / Roundup	135 mg/kg bw/d (maternal toxicity); not established for developmental effects	180 mg/kg bw/d (maternal toxicity); 91 mg/kg bw/d (developmental effects)	Mortality in pregnant dams; skeletal anomalies in fetuses ↑, delay in ossification	[REDACTED] 2003 ASB2012-11600

Studies with MON 0818 are of greater value for deriving the reference values because it cannot be excluded that the active ingredient or co-formulants other than the [REDACTED] to a certain degree may have contributed to the toxicity of Roundup. In the studies with oral administration of MON 0818, the lowest NOAELs were obtained in the Two-generation study by [REDACTED] (2007, ASB2010-365) and, with regard to maternal toxicity, in the developmental toxicity study by [REDACTED] (1990, ASB2009-9029). Numerically, both NOAELs were in the same magnitude of 10 – 12 mg/kg bw/day. The LOAEL of 9 mg/kg bw/day as calculated from the reproduction study with Roundup is in the same range. It is proposed to derive both the ADI and the AOEL on this basis. Since postnatal effects on pup survival in the reproduction study could be an acute effect and because a lower food consumption and body weight losses in pregnant dams in the developmental study at the

LOAEL were observed during the first days of treatment, a NOAEL of 10 – 12 mg/kg bw/day is also considered suitable for deriving the ARfD.

The toxic effects of the POE- [REDACTED] can be partly attributed to its irritating potential. However, systemic effects were also noted and, therefore, reduction of the usual safety factor is not feasible. When a safety factor of 100 is applied, a numeric value of 0.1 mg/kg bw/day for all three reference values (ADI, systemic AOEL, ARfD) will result.

In its recent evaluation, U.S. EPA has set a “chronic RfD” (ADI) and a reference value for “incidental oral (short-term and intermediate-term) exposure” (corresponding to the AOEL) of 0.15 mg/kg bw/day each (EPA, 2009, ASB2009-9022). However, these values are intended to be applied for the whole group of ethoxylated alkyl amines and are based on the NOAEL of 15 mg/kg bw/day as obtained in a 90-day rat study with ATMER®163. In this study, mortality occurred at the next higher dose level of 30 mg/kg bw/day [REDACTED] 1991, ASB2009-10488). ATMER®163 is an alkylamine formulation (CAS no. 70955-14-5) that is not contained as a co-formulant in plant protection products which are authorised in Germany. Therefore, this study is of no relevance for this evaluation.

The POE- [REDACTED] with CAS no. 61791-26-2 proved more toxic by the inhalation route than by oral intake. Therefore, setting of an inhalative AOEL as an additional reference value is needed. In the absence of an appropriate inhalative study with the surfactant itself, a calculation must be performed on the basis of a 4-week study with Roundup [REDACTED] 1983, TOX2002-694) under the (conservative) assumption that the effects were entirely due to the [REDACTED]. Based on the calculated NOAEL of 1.66 mg/kg bw/day for the [REDACTED] in this study (see section on short-term toxicity), an inhalative AOEL for this surfactant of 0.0166 mg/kg bw/day can be set when the safety factor of 100 is applied.

Dermal absorption

The POE- [REDACTED] with CAS no. 61791-26-2 is a surfactant that is used to enhance the uptake of the herbicidal compound glyphosate (or other herbicides) into the leaves or other part of the weeds which are intended to be controlled. Accordingly, it is a surface-active substance and a certain ability to penetrate through biological membranes can be assumed. However, estimation of dermal absorption for substances with strong irritating properties is difficult because dermal penetration can be either inhibited or facilitated.

Experimental data on dermal absorption is not available. Furthermore, there is no reliable data available that would allow “read-across”. Thus, the default values as proposed in the EFSA guidance document (EFSA, 2012, ASB2012-6959) should be used, depending on the concentration of the surfactant in the plant protection product.

Also if physico-chemical properties are taken into consideration, a high molecular weight of 928.31 D (EPA, 2009, ASB2009-9022) suggests poor dermal penetration. However, according to the EU guidance document on dermal absorption, the logP_{OW} of 3.15 does not allow reduction of the default values to 10 %.

Impact of POE- [REDACTED] on the toxicity of Roundup formulations

With regard to nearly all endpoints investigated, the POE- [REDACTED] (CAS no. 61791-26-2) was clearly more toxic than glyphosate. A direct comparison is shown in Table B.6.13-2. Data for the active substance have been taken from a recent evaluation (EU, 2001, ASB2009-4191).

Table B.6.13-2: Comparison of toxicity data for glyphosate and the POE- [REDACTED] surfactant with CAS no. 61791-26-2

End point	Glyphosate		POE- [REDACTED] surfactant	
Acute oral (rat)	LD ₅₀ >5000 mg/kg bw		LD ₅₀ : 864 mg/kg bw	
Acute dermal (rabbit)	LD ₅₀ >2000 mg/kg bw		LD ₅₀ >907 mg/kg bw	
Skin irritation	Not irritant		Irritant	
Eye irritation	Moderately to severely irritant		Severely irritant	
Skin sensitization	Negative		Sensitising	
Mutagenicity (gene mutations)	Negative		Negative	
Mutagenicity (chromosome aberrations)	Negative		Negative	
DNA damage	Negative		Equivocal (some evidence at high and clearly toxic doses)	
	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Short-term toxicity (rat, oral, 90 d)	150	300	20	60
Short-term toxicity (dog, oral, ca 3 mo)	300	1000	21	42
Reproduction toxicity (rat)	700 (parental) 2000 (repro) 700 (offspring)	2000 (parental) >2000 (repro) 2000 (offspring)	38 (parental) 12 (repro) 12 (offspring)	74 (parental) 38 (repro) 38 (offspring)
Developmental studies (rat), maternal toxicity	300	1000	10.8	72
Developmental studies (rat), fetal effects	300	1000	72	216

The higher toxicity of the surfactant might explain that also Roundup formulations when tested for different endpoints were more toxic than glyphosate ([REDACTED] 1982, TOX2002-693 and [REDACTED] 1983, TOX2002-694; [REDACTED] 2003, ASB2012-11600 and [REDACTED] 2007, ASB2012-2721). This is also the most likely explanation for poisoning incidents in humans by the oral or the inhalation route. A possible potentiation of toxicity of glyphosate IPA salt and the [REDACTED] in animals was suspected by [REDACTED] 1991, Z80636) who tested the acute oral toxicity of Roundup formulations in rats. However, taking into consideration the toxicological profile of glyphosate, synergism is not very likely. Most effects of both substances are different by nature. Even if the surfactant would enhance the oral absorption of glyphosate (usually about 30 % only), adverse effects are not expected because they occur only at exaggerated doses. The only effect for which dose additivity could be theoretically assumed is eye (and perhaps mucosal) irritation. However, the low acute oral toxicity and the high NOAELs of glyphosate in short-term oral studies (see Table B.6.13-2) suggest that the irritating potential of this active ingredient is not relevant after oral intake. Therefore, it is not very likely that glyphosate itself contributed that much to the toxicity of Roundup products in poisoning incidents in humans.

In sum, the available data is sufficient to support the assumption that critical effects of glyphosate-based PPP that were not seen with the active ingredient were due to toxicity of the POE- [REDACTED] surfactant alone.

B.6.14 Exposure data (Annex IIIA 7.3 to 7.5)

The review report for glyphosate 6511/VI/99-final – 21 January 2002 (ASB2009-4191) is considered to provide relevant basic information on risk assessment for glyphosate. But, new studies on the active substance glyphosate have been performed since then (see IIA 5 of the dossier for the active substance and chapter IIIA 7.6). Based on all available data, more appropriate values for the AOEL and dermal absorption have been derived (see Table B.6.14-1). The assessment presented below is based on these data.

Table B.6.14-1: Product information and toxicological reference values used for exposure assessment

Product	MON 52276
Formulation type	SL
Category	Herbicide
Container sizes, short description	1 L, 5 L, 10 L HDPE-containers with 63 mm openings, 20 L HDPE-containers with 61 mm openings and 60 – 1000 L drums
Active substances (incl. content)	Glyphosate (as its isopropylamine salt) 360 g/L (salt techn.: 486 g/L)
AOEL systemic	0.1 mg/kg bw/d
Inhalative absorption	100 %
Oral absorption	20 %
Dermal absorption	Concentrate: 0.1 % Dilution: 0.3 % (Dilution rate: 1:150) (SL-formulation containing 360 g/L glyphosate, MON 52276)

MON 52276 is used as a herbicide against annual, perennial and biennial weeds. It is applied pre- and post-planting, pre-emergence of crops or pre-harvest as well as post-emergence of weeds. Spray treatments are performed using tractor-mounted ground-boom sprayers and knapsack sprayers. A summary of the representative uses for MON 52276 is presented in Table B.6.14-2 below.

Table B.6.14-2: Summary of supported uses of MON 52276

Crops	F	Application rate per treatment		Spray volume [L/ha]	Maximum in-use concentration [kg a.s./hL]	Number of treatments min - max	Application technique	Acceptability of exposure assessment				
		[L product /ha]	[kg a.s./ha]					Operator GM	Operator UK	Worker	Bystander	Residents
All crops (pre-planting) ^{1), 6)}	F	1 – 6*	0.36 – 2.16	100 - 400	2.16	1 – 2*	Tractor-mounted ground boom sprayer with hydraulic nozzles					
All crops (pre-planting) ^{1), 7)}	F	1 – 6*	0.36 – 2.16	100 - 400	2.16	1 – 2*						
All crops (post-planting/pre-emergence of crops)	F	1 - 3	0.36 – 1.08	100 - 400	1.08	1						
Cereals, oil seeds ¹⁾ (both pre-harvest)	F	2 - 6	0.72 – 2.16	100 - 400	2.16	1						
Orchard crops, vines, incl. citrus & tree nuts ^{2), 3), 4), 5), 6)} (post emergence of weeds)	F	2 – 8*	0.72 – 2.88	100 - 400	2.88	1 – 3*	Knapsack sprayer					
Orchard crops, vines, incl. citrus & tree nuts ^{2), 3), 4), 5), 6)} (post emergence of weeds; spot treatment)	F	2 – 8*	0.72 – 2.88	100 - 400	2.88	1 – 3*						

F = field use

GM = German model

* Maximum dose per season not to exceed 4.32 kg a.s./ha

¹⁾ critical use for operators in low crops²⁾ critical use for operators during applications under high crops³⁾ critical use for bystanders⁴⁾ critical use for residents⁵⁾ critical use for workers

Since it could not be figured out unequivocally what is meant by 'all crops' by the applicant two different 'worst case' scenarios for residents are presented:

⁶⁾ Applications on lawn, pasture or meadow not included in 'all crops'⁷⁾ Applications on lawn, pasture or meadow included in 'all crops'

	Exposure acceptable without PPE / risk mitigation measures
	Exposure acceptable with PPE or risk mitigation measures required
	Exposure not acceptable/ Evaluation not possible
	No critical use

The results of exposure estimations for operators, bystanders, residents and workers are summarised in Table B.6.14-4, Table B.6.14-6, Table B.6.14-8 and Table B.6.14-10 below. Detailed estimations are provided in A.1.1, 1.2 and 1.3.

B.6.14.1 Operator exposure (IIIA 7.3.1 and 7.3.2)

B.6.14.1.1 Estimation of operator exposure

A summary of the exposure models used for estimation of operator exposure towards the active substances during application of MON 52276 according to intended uses is presented in Table B.6.14-3. Outcome of the estimation is presented in Table B.6.14-4. Detailed calculations are given in A 1.1.

Table B.6.14-3: Exposure models used for operator exposure estimations

Critical use	Cereals, oil seed rape, etc. (max. 6 L product/ha); Orchard crops, vines, incl. citrus & tree nuts (max. 8 L product/ha)
Model	German model [Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection), Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft, Berlin-Dahlem, Heft 277, 1992]
Critical use	Cereals, oil seed rape, etc. (max. 6 L product/ha); Orchard crops, vines, incl. citrus & tree nuts (max. 8 L product/ha)
Model	Revised UK-POEM [Estimation of Exposure and Absorption of Pesticides by Spray Operators, Scientific subcommittee on Pesticides and British Agrochemical Association Joint Medical Panel Report (UK MAFF), 1986 and the Predictive Operator Exposure Model (POEM) V 1.0, (UK MAFF), 1992]

Table B.6.14-4: Estimated operator exposure towards glyphosate from the use of MON 52276

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOEL
Tractor mounted boom spray applications outdoors to low crops Application rate: 2.16 kg a.s./ha			
German Model	no PPE ¹⁾	0.006245	6.2
Body weight: 70 kg	+ Gloves mixing/loading	0.004779	4.8
UK POEM	no PPE ²⁾	0.075	75.5
Application volume: 100 L/ha Container: 10 litres 63 mm closure Body weight: 60 kg	+ Gloves mixing/loading	0.067	66.9
Hand-held spray applications outdoors to high crops ³⁾ Application rate: 2.88 kg a.s./ha			
German Model	no PPE ¹⁾	0.0278	27.8
Body weight: 70 kg	+ Gloves mixing/loading	0.0195	19.5
Hand-held spray applications outdoors to low crops Application rate: 2.88 kg a.s./ha			
UK POEM	no PPE ²⁾	0.207	206.6
Application volume: 100 L/ha Container: 10 litres 63 mm closure Body weight: 60 kg	+ Gloves mixing/loading and application and impermeable coverall during application	0.085	84.7

¹⁾ no PPE: Operator wearing T-shirt and shorts

²⁾ no PPE: Operator wearing long sleeved shirt, long trousers ("permeable") but no gloves

³⁾ Since there are no data for outdoor applications of herbicides under high crops in the German model, this represents 'worst case'.

In conclusion, MON 52276 can be applied safely by operators using tractor-mounted and hand-held application techniques based on exposure calculations. Only in the case of hand-held spray applications using the UK POEM allocation of PPE to operators is necessary.

B.6.14.1.2 Measurement of operator exposure (mixer/loader/applicator) (IIIA 7.3.3)

Since the operator exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses (without PPE acc. to the German model, or if PPE is worn acc. to the UK POEM for hand-held applications), a study to provide measurements of operator exposure was not necessary and was therefore not performed..

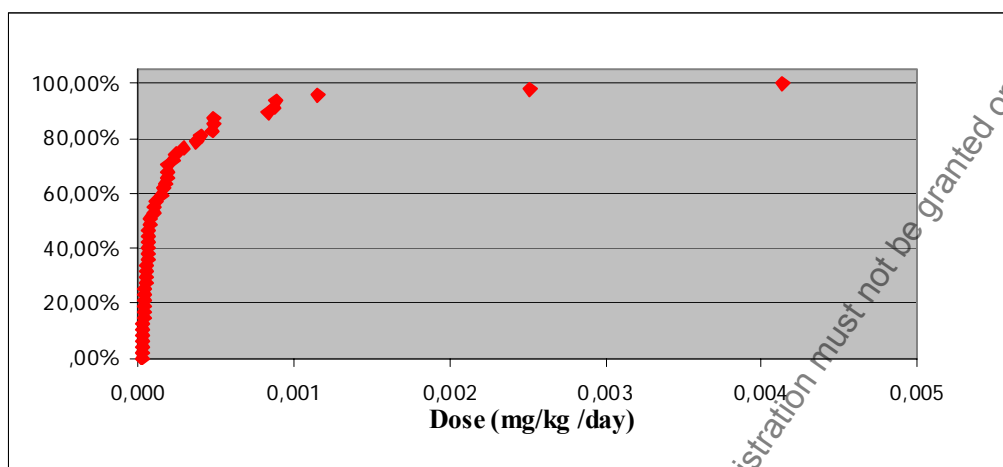
Nevertheless, the applicants submitted respective biomonitoring data from the Farm Family Exposure Study for farmers and their families published in the open literature (█ 2004; [ASB2012-11528](#)). This study was conducted in a southern (South Carolina) and northern (Minnesota) agricultural production area of the U.S.. The purpose of the study was to quantify actual internal pesticide exposure immediately before, during and after pesticide application.

For forty-eight farmer families, including 79 children, urine samples were collected for a 24-hour period prior to application, on the day of application and for three consecutive 24-hour periods thereafter. Urine specimens from 24-hour periods were mixed in such a way that the proportion of the individual volumes of the specimens remained unchanged in the composite mixture. Subsequently glyphosate concentrations were determined in the samples.

Farmers were not instructed or coached by the study investigators on how to apply the products. All farmers used tractor-mounted boom-sprayers and applied Roundup® Ultra (Monsanto Company) over glyphosate tolerant crops early in the growing season. About one-third of the farmers made applications on between 4 and 18 hectares, another third on 18–50 ha, and another third on 50–178 ha. Application rates were according to label recommendations. Sixty percent of applications were carried out using closed-cabs. 71 % of the farmers wore rubber gloves during application. 27 % of the farmers repaired their equipment during the application.

Glyphosate concentrations in the farmers' urine ranged from less than the limit of detection (LOD = 1 ppb) to a maximum of 233 ppb. Overall, only 60 % of the farmers showed detectable levels on the day of application, which further declined afterwards. Figure B.6.14-1 shows the cumulative frequency distribution of systemic doses obtained by the farmers.

Figure B.6.14-1: Systemic dose distribution for farmers applying glyphosate (Acquavella et al., 2004; ASB2012-11528)



The maximum estimated systemic dose for operators was 0.004 mg/kg/day, whereas the geometric mean was 0.0001 mg/kg bw/day.

B.6.14.2 Bystander and resident exposure (IIIA 7.4)

B.6.14.2.1 Estimation of bystander and resident exposure

Table B.6.14-5 shows the exposure model used for estimation of bystander and resident exposure towards glyphosate. Estimations are presented for adults as well as for children. In the case applications on lawn, on meadows and on pasture can be excluded, outcome of the 'worst case' estimations for applications in orchards etc. is presented in Table B.6.14-6. Detailed calculations are given in A.1.2.

If applications on lawn etc. have to be considered this scenario will represent 'worst case' conditions for residents. In this case it is referred to Table B.6.14-7 and Table B.6.14-8 and calculations in 0, too.

Table B.6.14-5: Exposure model used for bystander and resident exposure estimations

Critical use	Orchard crops, vines, incl. citrus & tree nuts (max. 3 x 8 L product/ha *)
Model	Martin, S. et al. (2008, ASB2009-450) [Guidance for Exposure and Risk Evaluation for Bystanders and Residents Exposed to Plant Protection Products During and After Application; J. Verbr. Lebensm. 3 (2008): 272-281 Birkhäuser Verlag Basel] and Bundesanzeiger (BAnz), 06 January 2012, Issue No. 4, pp. 75-76

* Maximum dose per season not to exceed 4.32 kg as/ha

Table B.6.14-6: Estimated bystander and resident exposure towards glyphosate from the use of MON 52276

	Glyphosate	
Model data	Total absorbed dose (mg/kg/day)	% of systemic AOEL
Hand-held spray application outdoors under high crops ('worst case') Application rate: 4.32 kg a.s./ha		
Bystanders (adult) Drift rate: 8.02 % ¹⁾ (3 m) Body weight: 60 kg	0.00135	1.35
Bystanders (children) Drift rate: 8.02 % ¹⁾ (3 m) Body weight: 16.15 kg	0.00133	1.33
Residents (adult) Drift rate: 7.23 % ²⁾ (3 m) Body weight: 60 kg	0.00039	0.39
Residents (children) Drift rate: 7.23 % ²⁾ (3 m) Body weight: 16.15 kg	0.00163	1.63

¹⁾ drift rate for ornamentals > 50 cm used as a default for herbicide applications under high crops

²⁾ 82th percentile for two applications

In the case applications on lawn are included in the term 'all crops' given by the applicant an alternative calculation for resident exposure is presented below.

Table B.6.14-7: Exposure model used for resident exposure estimations for applications on lawn

Critical use	Lawn, meadows, pasture (max. 2 x 6 L product/ha)
Model	Martin, S. et al. (2008, ASB2009-450) [Guidance for Exposure and Risk Evaluation for Bystanders and Residents Exposed to Plant Protection Products During and After Application; J. Verbr. Lebensm. 3 (2008): 272-281 Birkhäuser Verlag Basel] and Bundesanzeiger (BAnz), 06 January 2012, Issue No. 4, pp. 75-76

Table B.6.14-8: Estimated resident exposure towards glyphosate from the use of MON 52276 on lawn

	Glyphosate	
Model data	Total absorbed dose (mg/kg/day)	% of systemic AOEL
Tractor mounted spray application on lawn, pasture, meadow Application rate: 2 x 2.16 kg a.s./ha		
Bystanders (adult) Deposit: 100 % Body weight: 60 kg	0.00185	1.85
Bystanders (children) Deposit: 100 % Body weight: 16.15 kg	0.01598	16.0

B.6.14.2.2 Measurement of bystander or resident exposure

Since the bystander and resident exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses, a study to provide measurements of bystander or resident exposure was not necessary and was therefore not performed.

B.6.14.3 Worker exposure

For the intended uses of MON 52276 there are no foreseen re-entry activities. The only reasonable re-entry scenario is inspection of the crops. Furthermore, for spray treatments pre- and post-planting, and pre-emergence of the crops, as well as post-emergence of weeds in orchards, crop inspection activities normally require no dermal contact to the foliage, but rather consist of a visual inspection. Nevertheless, dermal contact with residues after application is considered as a 'worst case' scenario.

B.6.14.3.1 Estimation of worker exposure

Due to the low vapour pressure of glyphosate (1.31×10^{-5} Pa (25 °C)) respiratory exposure is considered irrelevant for re-entry tasks, so that exposure during these tasks occurs via skin contact with treated surfaces predominantly.

Table B.6.14-9 shows the exposure model used for estimation of dermal worker exposure after entry into a previously treated area or handling a crop treated with MON 52276 according to the critical use. Outcome of the estimation is presented in Table B.6.14-10. Detailed calculations are in A.1.3.

Table B.6.14-9: Exposure model for intended uses

Critical use	Orchard crops, vines, incl. citrus & tree nuts (max. 3 x 8 L product/ha *)
Model	German re-entry model, Krebs et al. (2000, TOX2004-1971) [Uniform Principles for Safeguarding the Health of Workers Re-entering Crop Growing Areas after Application of Plant Protection Products, Nachrichtenbl. Deut. Pflanzenschutzdienst., 52(1), p. 5-9]

* Maximum dose per season not to exceed 4.32 kg as/ha

Table B.6.14-10: Estimated worker exposure

		Glyphosate	
Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOED
Number of applications and application rate: 4.32 kg a.s./ha ¹⁾			
8 hours/day ²⁾ TC: 5000 cm ² /person/h ³⁾ DFR 1 µg/cm ² /kg a.s. ⁴⁾ Body weight: 60 kg	no PPE ⁵⁾	0.00864	8.6
	+ Gloves and protective suit	0.00043	0.4

¹⁾ Maximum dose per season; 'worst case' (no degradation between splitted dose applications taken into account)

²⁾ 8 h/day for professional applications; 'worst case', since normally there is no classical re-entry scenario for herbicidal applications under high crops

³⁾ Ornamentals, EUROPOEM II, 2002, Post-Application Exposure of Workers to Pesticides in Agriculture; default used for herbicidal applications under high crops

⁴⁾ no harmonised default is available, therefore default value acc. to the above mentioned model (even if a more conservative default value of 3 µg/cm²/kg a.s. is used, worker exposure is < 100 % of AOEL (26 % of AOEL))

⁵⁾ no PPE: Worker wearing long sleeved shirt, long trousers ("permeable") but no gloves

B.6.14.3.2 Measurement of worker exposure

Since the worker exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses, a study to provide measurements of worker exposure was not necessary and was therefore not performed.

Appendix 1 Exposure calculations

A 1.1 Operator exposure calculations (IIIA 7.3)

Table A 1: Input parameters considered for the estimation of operator exposure according to the German model (FCTM)

Formulation type:	SL		Application technique:	Field Crop Tractor Mounted (FCTM)	
Application rate (AR):	2.16	kg a.s./ha			
Area treated per day (A):	20	ha	Dermal hands m/l ($D_{M(H)}$):	2.4	mg/person/kg a.s.
Dermal absorption (DA):	0.1	% (concentr.)	Dermal hands appl. ($D_{A(H)}$):	0.38	mg/person/kg a.s.
	0.3	% (dilution)	Dermal body appl. ($D_{A(B)}$):	1.6	mg/person/kg a.s.
Inhalation absorption (IA):	100	%	Dermal head appl. ($D_{A(C)}$):	0.06	mg/person/kg a.s.
Body weight (BW):	70	kg/person	Inhalation m/l (I_m):	0.0006	mg/person/kg a.s.
AOEL	0.1	mg/kg bw/d	Inhalation appl. (I_A):	0.001	mg/person/kg a.s.

Table A 2: Estimation of operator exposure towards glyphosate using the German model

Without PPE			With PPE		
Operators: Systemic dermal exposure after application in cereals, oil seeds, pre-harvest; all crops, pre-planting					
<u>Dermal exposure during mixing/loading</u>					
Hands			Hands		
$SDE_{OM(H)} = (D_{M(H)} \times AR \times A \times DA) / BW$			$SDE_{OM(H)} = (D_{M(H)} \times AR \times A \times PPE^{1}) \times DA) / BW$		
$(2.4 \times 2.16 \times 20 \times 0.1 \%) / 70$			$(2.4 \times 2.16 \times 20 \times 0.01 \times 0.1 \%) / 70$		
External dermal exposure	103.68	mg/person	External dermal exposure	1.0368	mg/person
External dermal exposure	1.481143	mg/kg bw/d	External dermal exposure	0.014811	mg/kg bw/d
Systemic dermal exposure	0.001481	mg/kg bw/d	Systemic dermal exposure	0.000015	mg/kg bw/d
<u>Dermal exposure during application</u>					
Hands			Hands		
$SDE_{OA(H)} = (D_{A(H)} \times AR \times A \times DA) / BW$			$SDE_{OA(H)} = (D_{A(H)} \times AR \times A \times PPE \times DA) / BW$		
$(0.38 \times 2.16 \times 20 \times 0.3 \%) / 70$			$(0.38 \times 2.16 \times 20 \times 1 \times 0.3 \%) / 70$		
External dermal exposure	16.416	mg/person	External dermal exposure	16.416	mg/person
External dermal exposure	0.234514	mg/kg bw/d	External dermal exposure	0.234514	mg/kg bw/d
Systemic dermal exposure	0.000704	mg/kg bw/d	Systemic dermal exposure	0.000704	mg/kg bw/d
Body			Body		
$SDE_{OA(B)} = (D_{A(B)} \times AR \times A \times DA) / BW$			$SDE_{OA(B)} = (D_{A(B)} \times AR \times A \times PPE \times DA) / BW$		
$(1.6 \times 2.16 \times 20 \times 0.3 \%) / 70$			$(1.6 \times 2.16 \times 20 \times 1 \times 0.3 \%) / 70$		
External dermal exposure	69.12	mg/person	External dermal exposure	69.12	mg/person
External dermal exposure	0.987429	mg/kg bw/d	External dermal exposure	0.987429	mg/kg bw/d

Without PPE			With PPE		
Systemic dermal exposure	0.00296 2	mg/kg bw/d	Systemic dermal exposure	0.00296 2	mg/kg bw/d
Head			Head		
$SDE_{OA(C)} = (D_{A(C)} \times AR \times A \times DA) / BW$			$SDE_{OA(C)} = (D_{A(C)} \times AR \times A \times PPE \times DA) / BW$		
$(0.06 \times 2.16 \times 20 \times 0.3 \%) / 70$			$(0.06 \times 2.16 \times 20 \times 1 \times 0.3 \%) / 70$		
External dermal exposure	2.592	mg/person	External dermal exposure	2.592	mg/person
External dermal exposure	0.03702 9	mg/kg bw/d	External dermal exposure	0.03702 9	mg/kg bw/d
Systemic dermal exposure	0.00011 1	mg/kg bw/d	Systemic dermal exposure	0.00011 1	mg/kg bw/d
Total systemic dermal exposure: $SDE_o = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$			Total systemic dermal exposure: $SDE_o = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$		
Total external dermal exposure	191.808	mg/person	Total external dermal exposure	89.1648	mg/person
Total external dermal exposure	2.74011 4	mg/kg bw/d	Total external dermal exposure	1.27378 3	mg/kg bw/d
Total systemic dermal exposure	0.00525 8	mg/kg bw/d	Total systemic dermal exposure	0.00379 2	mg/kg bw/d
Operators: Systemic inhalation exposure after application in Cereals, oil seeds, pre-harvest; all crops, pre-planting					
<u>Inhalation exposure during mixing/loading</u>					
$SIE_{OM} = (I_M \times AR \times A \times IA) / BW$			$SIE_{OM} = (I_M \times AR \times A \times PPE \times IA) / BW$		
$(0.0006 \times 2.16 \times 20 \times 100 \%) / 70$			$(0.0006 \times 2.16 \times 20 \times 1 \times 100 \%) / 70$		
External inhalation exposure	0.02592	mg/person	External inhalation exposure	0.02592	mg/person
External inhalation exposure	0.00037	mg/kg bw/d	External inhalation exposure	0.00037	mg/kg bw/d
Systemic inhalation exposure	0.00037	mg/kg bw/d	Systemic inhalation exposure	0.00037	mg/kg bw/d
<u>Inhalation exposure during application</u>					
$SIE_{OA} = (I_A \times AR \times A \times IA) / BW$			$SIE_{OA} = (I_A \times AR \times A \times PPE \times IA) / BW$		
$(0.001 \times 2.16 \times 20 \times 100 \%) / 70$			$(0.001 \times 2.16 \times 20 \times 1 \times 100 \%) / 70$		
External inhalation exposure	0.0432	mg/person	External inhalation exposure	0.0432	mg/person
External inhalation exposure	0.00061 7	mg/kg bw/d	External inhalation exposure	0.00061 7	mg/kg bw/d
Systemic inhalation exposure	0.00061 7	mg/kg bw/d	Systemic inhalation exposure	0.00061 7	mg/kg bw/d
Total systemic inhalation exposure: $SIE_o = SIE_{OM} + SIE_{OA}$			Total systemic inhalation exposure: $SIE_o = SIE_{OM} + SIE_{OA}$		
Total external inhalation exposure	0.06912	mg/person	Total external inhalation exposure	0.06912	mg/person
Total external inhalation exposure	0.00098 7	mg/kg bw/d	Total external inhalation exposure	0.00098 7	mg/kg bw/d
Total systemic inhalation exposure	0.00098 7	mg/kg bw/d	Total systemic inhalation exposure	0.00098 7	mg/kg bw/d
Total systemic exposure: $SE_o = SDE_o + SIE_o$			Total systemic exposure: $SE_o = SDE_o + SIE_o$		
Total systemic exposure	0.43718 4	mg/person	Total systemic exposure	0.33454 1	mg/person
Total systemic exposure	0.00624 5	mg/kg bw/d	Total systemic exposure	0.00477 9	mg/kg bw/d
% of AOEL	6.2	%	% of AOEL	4.8	%
Reduction factor for gloves is 0.01 (professional appl.)					

Table A 3: Input parameters considered for the estimation of operator exposure according to the German model (HCHH)

Formulation type:	SL		Application technique:	High Crop Hand Held (HCHH)	
Application rate (AR):	2.88	kg a.s./ha			
Area treated per day (A):	1	ha	Dermal hands m/l ($D_{M(H)}$):	205	mg/person/kg a.s.
Dermal absorption (DA):	0.1	% (concentr.)	Dermal hands appl. ($D_{A(H)}$):	10.6	mg/person/kg a.s.
	0.3	% (dilution)	Dermal body appl. ($D_{A(B)}$):	25	mg/person/kg a.s.
Inhalation absorption (IA):	100	%	Dermal head appl. ($D_{A(C)}$):	4.8	mg/person/kg a.s.
Body weight (BW):	70	kg/person	Inhalation m/l (I_M):	0.05	mg/person/kg a.s.
AOEL	0.1	mg/kg bw/d	Inhalation appl. (I_A):	0.3	mg/person/kg a.s.

Table A 4: Estimation of operator exposure towards glyphosate using the German model (HCHH)

Without PPE			With PPE		
Operators: Systemic dermal exposure after application in grapevine, orchard crops, tree nuts					
<u>Dermal exposure during mixing/loading</u>					
Hands			Hands		
$SDE_{OM(H)} = (D_{M(H)} \times AR \times A \times DA) / BW$			$SDE_{OM(H)} = (D_{M(H)} \times AR \times A \times PPE^{1)} \times DA) / BW$		
$(205 \times 2.88 \times 1 \times 0.1 \%) / 70$			$(205 \times 2.88 \times 1 \times 0.01 \times 0.1 \%) / 70$		
External dermal exposure	590.4	mg/person	External dermal exposure	5.904	mg/person
External dermal exposure	8.434286	mg/kg bw/d	External dermal exposure	0.084343	mg/kg bw/d
Systemic dermal exposure	0.008434	mg/kg bw/d	Systemic dermal exposure	0.000084	mg/kg bw/d
<u>Dermal exposure during application</u>					
Hands			Hands		
$SDE_{OA(H)} = (D_{A(H)} \times AR \times A \times DA) / BW$			$SDE_{OA(H)} = (D_{A(H)} \times AR \times A \times PPE \times DA) / BW$		
$(10.6 \times 2.88 \times 1 \times 0.3 \%) / 70$			$(10.6 \times 2.88 \times 1 \times 1 \times 0.3 \%) / 70$		
External dermal exposure	30.528	mg/person	External dermal exposure	30.528	mg/person
External dermal exposure	0.436114	mg/kg bw/d	External dermal exposure	0.436114	mg/kg bw/d
Systemic dermal exposure	0.001308	mg/kg bw/d	Systemic dermal exposure	0.001308	mg/kg bw/d
Body			Body		
$SDE_{OA(B)} = (D_{A(B)} \times AR \times A \times DA) / BW$			$SDE_{OA(B)} = (D_{A(B)} \times AR \times A \times PPE \times DA) / BW$		
$(25 \times 2.88 \times 1 \times 0.3 \%) / 70$			$(25 \times 2.88 \times 1 \times 1 \times 0.3 \%) / 70$		
External dermal exposure	72	mg/person	External dermal exposure	72	mg/person
External dermal exposure	1.028571	mg/kg bw/d	External dermal exposure	1.028571	mg/kg bw/d
Systemic dermal exposure	0.003086	mg/kg bw/d	Systemic dermal exposure	0.003086	mg/kg bw/d
Head			Head		
$SDE_{OA(C)} = (D_{A(C)} \times AR \times A \times DA) / BW$			$SDE_{OA(C)} = (D_{A(C)} \times AR \times A \times PPE \times DA) / BW$		
$(4.8 \times 2.88 \times 1 \times 0.3 \%) / 70$			$(4.8 \times 2.88 \times 1 \times 1 \times 0.3 \%) / 70$		
External dermal exposure	13.824	mg/person	External dermal exposure	13.824	mg/person

Without PPE			With PPE		
exposure					
External dermal exposure	0.197486	mg/kg bw/d	External dermal exposure	0.197486	mg/kg bw/d
Systemic dermal exposure	0.000592	mg/kg bw/d	Systemic dermal exposure	0.000592	mg/kg bw/d
Total systemic dermal exposure: $SDE_O = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$			Total systemic dermal exposure: $SDE_O = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$		
Total external dermal exposure	706.752	mg/person	Total external dermal exposure	122.256	mg/person
Total external dermal exposure	10.096457	mg/kg bw/d	Total external dermal exposure	1.746514	mg/kg bw/d
Total systemic dermal exposure	0.013421	mg/kg bw/d	Total systemic dermal exposure	0.005091	mg/kg bw/d
Operators: Systemic inhalation exposure after application in Grapevine, orchard crops, tree nuts					
<u>Inhalation exposure during mixing/loading</u>					
$SIE_{OM} = (I_M \times AR \times A \times IA) / BW$			$SIE_{OM} = (I_M \times AR \times A \times PPE \times IA) / BW$		
$(0.05 \times 2.88 \times 1 \times 100 \%) / 70$			$(0.05 \times 2.88 \times 1 \times 1 \times 100 \%) / 70$		
External inhalation exposure	0.144	mg/person	External inhalation exposure	0.144	mg/person
External inhalation exposure	0.002057	mg/kg bw/d	External inhalation exposure	0.002057	mg/kg bw/d
Systemic inhalation exposure	0.002057	mg/kg bw/d	Systemic inhalation exposure	0.002057	mg/kg bw/d
<u>Inhalation exposure during application</u>					
$SIE_{OA} = (I_A \times AR \times A \times IA) / BW$			$SIE_{OA} = (I_A \times AR \times A \times PPE \times IA) / BW$		
$(0.3 \times 2.88 \times 1 \times 100 \%) / 70$			$(0.3 \times 2.88 \times 1 \times 1 \times 100 \%) / 70$		
External inhalation exposure	0.864	mg/person	External inhalation exposure	0.864	mg/person
External inhalation exposure	0.012343	mg/kg bw/d	External inhalation exposure	0.012343	mg/kg bw/d
Systemic inhalation exposure	0.012343	mg/kg bw/d	Systemic inhalation exposure	0.012343	mg/kg bw/d
Total systemic inhalation exposure: $SIE_O = SIE_{OM} + SIE_{OA}$			Total systemic inhalation exposure: $SIE_O = SIE_{OM} + SIE_{OA}$		
Total external inhalation exposure	1.008	mg/person	Total external inhalation exposure	1.008	mg/person
Total external inhalation exposure	0.0144	mg/kg bw/d	Total external inhalation exposure	0.0144	mg/kg bw/d
Total systemic inhalation exposure	0.0144	mg/kg bw/d	Total systemic inhalation exposure	0.0144	mg/kg bw/d
Total systemic exposure: $SE_O = SDE_O + SIE_O$			Total systemic exposure: $SE_O = SDE_O + SIE_O$		
Total systemic exposure	1.947456	mg/person	Total systemic exposure	1.36296	mg/person
Total systemic exposure	0.027821	mg/kg bw/d	Total systemic exposure	0.019471	mg/kg bw/d
% of AOEL	27.8	%	% of AOEL	19.5	%
¹⁾ reduction factor for gloves is 0.01 (professional appl.)					

Table A 5: Estimation of operator exposure towards glyphosate using the UK-POEM (FCTM)

Without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Active substance	Glyphosate			
Product	MON 52276			
Formulation type	water-based			
Concentration of a.s.	360	mg/mL		
Dose	6	L preparation/ha	(2.16 kg a.s./ha)	
Application volume	100	L/ha		
Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Container	10 litres 63 mm closure			
Work rate/day	50	ha		
Duration of spraying	6	h		
PPE during mix./loading	None			
PPE during application	None			
Dermal absorption from product	0.1	%		
Dermal absorption from spray	0.3	%		
EXPOSURE DURING MIXING AND LOADING				
Container size	10	Litres		
Hand contamination/operation	0,05	mL		
Application dose	6	Litres product/ha		
Work rate	50	ha/day		
Number of operations	30	/day		
Hand contamination	1.5	mL/day		
Protective clothing	None			
Transmission to skin	100	%		
Dermal exposure to formulation	1.5	mL/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	100	spray/ha		
Volume of surface contamination	10	mL/h		
Distribution	Hands	Trunk	Legs	
	65 %	10 %	25 %	
Clothing	None	Permeable	Permeable	
Penetration	100 %	5 %	15 %	
Dermal exposure	6.5	0.05	0.375	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	mL/day		
ABSORBED DERMAL DOSE				
	Mix/load		Application	
Dermal exposure	1.5	mL/day	41.55	mL/day
Concen. of a.s. product or spray	360	mg/mL	21.6	mg/mL
Dermal exposure to a.s.	540	mg/day	897.48	mg/day
Percent absorbed	0.1	%	0.3	%
Absorbed dose	0.54	mg/day	2.692	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	mL/h		
Duration of exposure	6	h		
Concentration of a.s. in spray	21.6	mg/mL		
Inhalation exposure to a.s.	1.296	mg/day		
Percent absorbed	100	%		
Absorbed dose	1.296	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	4.528	mg/day		
Operator body weight	60	kg		

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Operator exposure	0.075	mg/kg bw/day		
Amount of AOEL	75.5	%		

With PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Active substance	Glyphosate			
Product	MON 52276			
Formulation type	water-based			
Concentration of a.s.	360	mg/mL		
Dose	6	L preparation/ha	(2.16 kg a.s./ha)	
Application volume	100	L/ha		
Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Container	10 litres 63 mm closure			
Work rate/day	50	ha		
Duration of spraying	6	h		
PPE during mix./loading	Gloves			
PPE during application	None			
Dermal absorption from product	0.1	%		
Dermal absorption from spray	0.3	%		
EXPOSURE DURING MIXING AND LOADING				
Container size	10	Litres		
Hand contamination/operation	0.05	mL		
Application dose	6	Litres product/ha		
Work rate	50	ha/day		
Number of operations	30	/day		
Hand contamination	1.5	mL/day		
Protective clothing	Gloves			
Transmission to skin	5	%		
Dermal exposure to formulation	0.075	mL/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	100	spray/ha		
Volume of surface contamination	10	mL/h		
Distribution	Hands	Trunk	Legs	
	65 %	10 %	25 %	
Clothing	None	Permeable	Permeable	
Penetration	100 %	5 %	15 %	
Dermal exposure	6.5	0.05	0.375	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	mL/day		
ABSORBED DERMAL DOSE				
	Mix/load		Application	
Dermal exposure	0.075	mL/day	41.55	mL/day
Concen. of a.s. product or spray	360	mg/mL	21.6	mg/mL
Dermal exposure to a.s.	27	mg/day	897.48	mg/day
Percent absorbed	0.1	%	0.3	%
Absorbed dose	0.027	mg/day	2.692	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	mL/h		
Duration of exposure	6	h		
Concentration of a.s. in spray	21.6	mg/mL		
Inhalation exposure to a.s.	1.296	mg/day		
Percent absorbed	100	%		
Absorbed dose	1.296	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	4.015	mg/day		

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Operator body weight	60	kg		
Operator exposure	0.067	mg/kg bw/day		
Amount of AOEL	66.9	%		

Table A 6: Estimation of operator exposure towards glyphosate using the UK-POEM (Hand-held, 15 L tank, downwards)

Without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Active substance	Glyphosate			
Product	MON 52276			
Formulation type	water-based			
Concentration of a.s.	360	mg/mL		
Dose	8	L preparation/ha	(2.88 kg a.s./ha)	
Application volume	100	L/ha		
Application method	Hand-held sprayer (15 L tank): hydraulic nozzles. Outdoor, low level target			
Container	10 litres 63 mm closure			
Work rate/day	1	ha		
Duration of spraying	6	h		
PPE during mix./loading	None			
PPE during application	None			
Dermal absorption from product	0.1	%		
Dermal absorption from spray	0.3	%		
EXPOSURE DURING MIXING AND LOADING				
Container size	10	Litres		
Hand contamination/operation	0.05	mL		
Application dose	8	Litres product/ha		
Work rate	1	ha/day		
Number of operations	7	/day		
Hand contamination	0.35	mL/day		
Protective clothing	None			
Transmission to skin	100	%		
Dermal exposure to formulation	0.35	mL/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Hand-held sprayer (15 L tank): hydraulic nozzles. Outdoor, low level target			
Application volume	100	spray/ha		
Volume of surface contamination	50	mL/h		
Distribution	Hands	Trunk	Legs	
	25 %	25 %	50 %	
Clothing	None	Permeable	Permeable	
Penetration	100 %	20 %	18 %	
Dermal exposure	10	2.5	4.5	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	mL/day		
ABSORBED DERMAL DOSE				
	Mix/load		Application	
Dermal exposure	0.35	mL/day	102	mL/day
Concen. of a.s. product or spray	360	mg/mL	28.8	mg/mL
Dermal exposure to a.s.	126	mg/day	2937.6	mg/day
Percent absorbed	0.1	%	0.3	%
Absorbed dose	0.126	mg/day	8.813	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.02	mL/h		

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Duration of exposure	6	h		
Concentration of a.s. in spray	28.8	mg/mL		
Inhalation exposure to a.s.	3.456	mg/day		
Percent absorbed	100	%		
Absorbed dose	3.456	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	12.395	mg/day		
Operator body weight	60	kg		
Operator exposure	0.207	mg/kg bw/day		
Amount of AOEL	206.6	%		

With PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Active substance	Glyphosate			
Product	MON 52276			
Formulation type	water-based			
Concentration of a.s.	360	mg/mL		
Dose	8	L preparation/ha	(2.88 kg a.s./ha)	
Application volume	100	L/ha		
Application method	Hand-held sprayer (15 L tank): hydraulic nozzles. Outdoor, low level target			
Container	10 litres 63 mm closure			
Work rate/day	1	ha		
Duration of spraying	6	h		
PPE during mix./loading	Gloves			
PPE during application	Gloves and impermeable coveralls			
Dermal absorption from product	0.1	%		
Dermal absorption from spray	0.3	%		
EXPOSURE DURING MIXING AND LOADING				
Container size	10	Litres		
Hand contamination/operation	0.05	mL		
Application dose	8	Litres product/ha		
Work rate	1	ha/day		
Number of operations	4	/day		
Hand contamination	0.35	mL/day		
Protective clothing	Gloves			
Transmission to skin	5	%		
Dermal exposure to formulation	0.018	mL/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Hand-held sprayer (15 L tank): hydraulic nozzles. Outdoor, low level target			
Application volume	100	spray/ha		
Volume of surface contamination	50	mL/h		
Distribution	Hands	Trunk	Legs	
	25 %	25 %	50 %	
Clothing	Gloves	Impermeable	Impermeable	
Penetration	10 %	5 %	5 %	
Dermal exposure	1.25	0.625	1.25	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	18.75	mL/day		
ABSORBED DERMAL DOSE				
	Mix/load		Application	
Dermal exposure	0.018	mL/day	18.75	mL/day
Concen. of a.s. product or spray	360	mg/mL	28.8	mg/mL
Dermal exposure to a.s.	6.3	mg/day	540	mg/day
Percent absorbed	0.1	%	0.3	%

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)				
Absorbed dose	0.006	mg/day	1.62	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.02	mL/h		
Duration of exposure	6	h		
Concentration of a.s. in spray	28.8	mg/mL		
Inhalation exposure to a.s.	3.456	mg/day		
Percent absorbed	100	%		
Absorbed dose	3.456	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	5.082	mg/day		
Operator body weight	60	kg		
Operator exposure	0.085	mg/kg bw/day		
Amount of AOEL	84.7	%		

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

A 1.2 Bystander and resident exposure calculations (IIIA 7.4)

Table A 7: Input parameters considered for the estimation of bystander exposure

Intended use::	All high crops intended		Drift (D) ¹⁾ :	8.02	% (HC, 3 m)
Application rate (AR):	2.88	kg a.s./ha	Exposed body surface area (BSA):	1	m ² (adults)
	288	mg/m ²		0.21	m ² (children)
Body weight (BW):	60	kg/person (adults)	Specific Inhalation Exposure (I* _A):	0.3	mg/kg a.s. (6 hours, adults)
	16.15	kg/person (children)		0.172414	mg/kg a.s. (6 hours, children)
Dermal absorption (DA):	0.3	% ('worst case')	Area Treated (A):	1	ha/d (based on HCHH)
Inhalation absorption (IA):	100	%			
AOEL:	0.1	mg/kg bw/d	Exposure duration (T):	5	min

¹⁾ drift rate for ornamentals > 50 cm used as a default for herbicidal applications under high crops

Table A 8: Estimation of bystander exposure towards glyphosate

Adults			Children		
Bystander: Systemic dermal exposure during/after application under high crops (via spray drift)					
$SDE_B = (AR \times D \times BSA \times DA) / BW$			$SDE_B = (AR \times D \times BSA \times DA) / BW$		
$(288 \times 8.02 \% \times 1 \times 0.3 \%) / 60$			$(288 \times 8.02 \% \times 0.21 \times 0.3 \%) / 16.15$		
External dermal exposure	23.0976	mg/person	External dermal exposure	4.850496	mg/person
External dermal exposure	0.38496	mg/kg bw/d	External dermal exposure	0.30034	mg/kg bw/d
Systemic dermal exposure	0.001155	mg/kg bw/d	Systemic dermal exposure	0.000901	mg/kg bw/d
Bystander: Systemic inhalation exposure during/after application under high crops (via spray drift)					
$SIE_B = (I^*_A \times AR \times A \times T \times IA) / BW$			$SIE_B = (I^*_A \times AR \times A \times T \times IA) / BW$		
$(0.3 / 360 \times 2.88 \times 1 \times 5 \times 100 \%) / 60$			$(0.172414 / 360 \times 2.88 \times 1 \times 5 \times 100 \%) / 16.15$		
External inhalation exposure	0.012	mg/person	External inhalation exposure	0.006897	mg/person
External inhalation exposure	0.0002	mg/kg bw/d	External inhalation exposure	0.000427	mg/kg bw/d
Systemic inhalation exposure	0.0002	mg/kg bw/d	Systemic inhalation exposure	0.000427	mg/kg bw/d
Total systemic exposure: $SE_B = SDE_B + SIE_B$			Total systemic exposure: $SE_B = SDE_B + SIE_B$		
Total systemic exposure	0.081293	mg/person	Total systemic exposure	0.021448	mg/person
Total systemic exposure	0.001355	mg/kg bw/d	Total systemic exposure	0.001328	mg/kg bw/d
% of AOEL	1.35	%	% of AOEL	1.33	%

Table A 9: Input parameters considered for the estimation of resident exposure ('worst case' if no applications on lawn, pasture and meadow are intended)

Intended uses:	All high crops intended		Drift (D) ²⁾ :	7.23	% (HC, 3 m)
Application rate (AR): ¹⁾	4.32	kg a.s./ha	Transfer coefficient (TC):	7300	cm ² /h (adults)
	0.0432	mg/cm ²		2600	cm ² /h (children)
Number of applications (NA):	2		Turf Transferable Residues (TTR):	5	%
Body weight (BW):	60	kg/person (adults)	Exposure Duration (H):	2	h
	16.15	kg/person (children)	Airborne Concentration of Vapour (ACV):	0.001	mg/m ³
Dermal absorption (DA):	0.3	% ('worst case')	Inhalation Rate (IR):	16.57	m ³ /d (adults)
Inhalation absorption (IA):	100	%		8.31	m ³ /d (children)
Oral absorption (OA):	20	%	Saliva Extraction Factor (SE):	50	%
AOEL:	0.1	mg/kg bw/d	Surface Area of Hands (SA):	20	cm ²
			Frequency of Hand to Mouth (Freq):	20	events/h
			Dislodgeable foliar residues (DFR):	20	%
			Ingestion Rate for Mouthing of Grass/Day (IGR):	25	cm ² /d

¹⁾ maximum dose per season

²⁾ drift rate for ornamentals > 50 cm used as a default for herbicidal applications under high crops, 82 th percentile for 2 applications

Table A 10: Estimation of resident exposure towards glyphosate ('worst case' if no applications on lawn, pasture and meadow are intended)

Adults			Children		
Residents: Systemic dermal exposure after application under high crops (via deposits caused by spray drift)					
$SDE_R = (AR \times NA \times D \times TTR \times TC \times H \times DA) / BW$			$SDE_R = (AR \times NA \times D \times TTR \times TC \times H \times DA) / BW$		
$(0.0432 \times 7.23 \% \times 5 \% \times 7300 \times 2 \times 0.3 \%) / 60$			$(0.0432 \times 1 \times 7.23 \% \times 5 \% \times 2600 \times 2 \times 0.3 \%) / 16.15$		
External dermal exposure	2.280053	mg/person	External dermal exposure	0.812074	mg/person
External dermal exposure	0.038001	mg/kg bw/d	External dermal exposure	0.050283	mg/kg bw/d
Systemic dermal exposure	0.000114	mg/kg bw/d	Systemic dermal exposure	0.000151	mg/kg bw/d
Residents: Systemic inhalation exposure after application under high crops (via vapour)					
$SIE_R = (AC_V \times IR \times IA) / BW$			$SIE_R = (AC_V \times IR \times IA) / BW$		
$(0.001 \times 16.57 \times 100 \%) / 60$			$(0.001 \times 8.31 \times 100 \%) / 16.15$		
External inhalation exposure	0.01657	mg/person	External inhalation exposure	0.00831	mg/person
External inhalation exposure	0.000276	mg/kg bw/d	External inhalation exposure	0.000515	mg/kg bw/d
Systemic inhalation exposure	0.000276	mg/kg bw/d	Systemic inhalation exposure	0.000515	mg/kg bw/d
			Residents: Systemic oral exposure (hand-to-mouth transfer)		
			$SOE_{R(H)} = (AR \times NA \times D \times TTR \times SE \times SA \times Freq \times H \times OA) / BW$		
			$(0.0432 \times 1 \times \% \times 5 \% \times 50 \% \times 20 \times 20 \times 2 \times 20 \%) / 16.15$		
			External oral exposure	0.062467	mg/person
			External oral exposure	0.003868	mg/kg bw/d
			Systemic oral exposure	0.000774	mg/kg bw/d
			Residents: Systemic oral exposure (object-to-mouth transfer)		
			$SOE_{R(O)} = (AR \times NA \times D \times DFR \times IgR \times OA) / BW$		
			$(0.0432 \times 1 \times \% \times 20 \% \times 25 \times 20 \%) / 16.15$		
			External oral exposure	0.015617	mg/person
			External oral exposure	0.000967	mg/kg bw/d
			Systemic oral exposure	0.000193	mg/kg bw/d
Total systemic exposure: $SE_R = SDE_R + SIE_R$			Total systemic exposure: $SE_R = SDE_R + SIE_R + SOE_{R(H)} + SOE_{R(O)}$		
Total systemic exposure	0.02341	mg/person	Total systemic exposure	0.026363	mg/person
Total systemic exposure	0.00039	mg/kg bw/d	Total systemic exposure	0.001632	mg/kg bw/d
% of AOEL	0.39	%	% of AOEL	1.63	%

Table A 11: Input parameters considered for the estimation of resident exposure ('worst case' if applications on lawn, pasture and meadow are intended)

Intended uses:	Pastures, lawn, meadow		Deposit (D):	100	
Application rate (AR):	2.16	kg a.s./ha	Transfer coefficient (TC):	7300	cm ² /h (adults)
	0.0216	mg/cm ²		2600	cm ² /h (children)
Number of applications (NA):	2		Turf Transferable Residues (TTR):	5	%
Body weight (BW):	60	kg/person (adults)	Exposure Duration (H):	2	h
	16.15	kg/person (children)	Airborne Concentration of Vapour (ACV):	0.001	mg/m ³
Dermal absorption (DA):	0.3	% ('worst case')	Inhalation Rate (IR):	16.57	m ³ /d (adults)
Inhalation absorption (IA):	100	%		8.31	m ³ /d (children)
Oral absorption (OA):	20	%	Saliva Extraction Factor (SE):	50	%
AOEL:	0.1	mg/kg bw/d	Surface Area of Hands (SA):	20	cm ²
			Frequency of Hand to Mouth (Freq):	20	events/h
			Dislodgeable foliar residues (DFR):	20	%
			Ingestion Rate for Mouthing of Grass/Day (IgR):	25	cm ² /d

Table A 12: Estimation of resident exposure towards glyphosate ('worst case' if applications on lawn, pasture and meadow are intended)

Adults			Children		
Residents: Systemic dermal exposure after application on pastures, lawn, meadow (via deposits caused by spray drift)					
$SDE_R = (AR \times NA \times D \times TTR \times TC \times H \times DA) / BW$			$SDE_R = (AR \times NA \times D \times TTR \times TC \times H \times DA) / BW$		
$(0.0216 \times 2 \times 100\% \times 5\% \times 7300 \times 2 \times 0.3\%) / 60$			$(0.0216 \times 2 \times 100\% \times 5\% \times 2600 \times 2 \times 0.3\%) / 16.15$		
External dermal exposure	31.536	mg/person	External dermal exposure	11.232	mg/person
External dermal exposure	0.5256	mg/kg bw/d	External dermal exposure	0.69548	mg/kg bw/d
Systemic dermal exposure	0.001577	mg/kg bw/d	Systemic dermal exposure	0.002086	mg/kg bw/d
Residents: Systemic inhalation exposure after application on pastures, lawn, meadow (via vapour)					
$SIE_R = (AC_V \times IR \times IA) / BW$			$SIE_R = (AC_V \times IR \times IA) / BW$		
$(0.001 \times 16.57 \times 100\%) / 60$			$(0.001 \times 8.31 \times 100\%) / 16.15$		
External inhalation exposure	0.01657	mg/person	External inhalation exposure	0.00831	mg/person
External inhalation exposure	0.000276	mg/kg bw/d	External inhalation exposure	0.000515	mg/kg bw/d
Systemic inhalation exposure	0.000276	mg/kg bw/d	Systemic inhalation exposure	0.000515	mg/kg bw/d
			Residents: Systemic oral exposure (hand-to-mouth transfer)		
			$SOE_{R(H)} = (AR \times NA \times D \times TTR \times SE \times SA \times Freq \times H \times OA) / BW$		
			$(0.0216 \times 2 \times \% \times 5\% \times 50\% \times 20 \times 20 \times 2 \times 20\%) / 16.15$		
			External oral exposure	0.864	mg/person
			External oral exposure	0.053498	mg/kg bw/d
			Systemic oral exposure	0.0107	mg/kg bw/d
			Residents: Systemic oral exposure (object-to-mouth transfer)		
			$SOE_{R(O)} = (AR \times NA \times D \times DFR \times IgR \times OA) / BW$		
			$(0.0216 \times 2 \times \% \times 20\% \times 25 \times 20\%) / 16.15$		
			External oral exposure	0.216	mg/person
			External oral exposure	0.013375	mg/kg bw/d
			Systemic oral exposure	0.002675	mg/kg bw/d
Total systemic exposure: $SE_R = SDE_R + SIE_R$			Total systemic exposure: $SE_R = SDE_R + SIE_R + SOE_{R(H)} + SOE_{R(O)}$		
Total systemic exposure	0.111178	mg/person	Total systemic exposure	0.258006	mg/person
Total systemic exposure	0.001853	mg/kg bw/d	Total systemic exposure	0.015976	mg/kg bw/d
% of AOEL	1.85	%	% of AOEL	15.98	%

A 1.3 Worker exposure calculations (IIIA 7.5)

Table A 13: Input parameters considered for the estimation of worker exposure

Intended use(s):	All high crops intended	Dislodgeable foliar residues (DFR):	1	µg/cm ² /kg a.s.
Application rate (AR):	4.32 ¹⁾	kg a.s./ha	Transfer coefficient (TC):	5000
Number of applications (NA):			Work rate per day (WR):	8
Body weight (BW):	60	kg/person	PPE	5
Dermal absorption (DA):	0.3	% ('worst case')		
AOEL	0.1	mg/kg bw/d		

¹⁾ Maximum dose per season, therefore no number of applications considered; no degradation between splitted dose applications taken into account

Table A 14: Estimation of worker exposure towards glyphosate using the German re-entry model

Without PPE ¹⁾			With PPE ²⁾		
Worker (re-entry): Systemic dermal exposure after application under high crops					
SDE _w = (DFR x TC x WR x AR x DA) / BW			SDE _w = (DFR x TC x WR x AR x PPE x DA) / BW		
(1 x 5000 x 8 x 4.32 x x 0.3 %) / 60			(1 x 5000 x 8 x 4.32 x x 5 % x 0.3 %) / 60		
External dermal exposure	172.8	mg/person	External dermal exposure	8.64	mg/person
External dermal exposure	2.88	mg/kg bw/d	External dermal exposure	0.144	mg/kg bw/d
Total systemic exposure	0.5184	mg/person	Total systemic exposure	0.02592	mg/person
Total systemic exposure	0.00864	mg/kg bw/d	Total systemic exposure	0.000432	mg/kg bw/d
% of AOEL	8.6	%	% of AOEL	0.4	%
1) acceptable without PPE: Treated areas/crops may not be entered until the spray coating has dried					
2) acceptable only with PPE: Re-entering the treated areas/crops is only possible on the day of application wearing personal protective equipment which is specified for applying the particular product. Successive work on/in treated areas/crops may fundamentally not be carried out until 24 hours after applying the product. Within 48 hours, protective suits against pesticides and standard protective gloves (plant protection) are to be worn.					

B.6.15 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5 KIIIA1 7 (OECD)	EFSA	2009	Reasoned opinion: Modification of the residue definition of Glyphosate in genetically modified maize grain and soybeans, and in products of animal origin EFSA Journal 2009; 7(9):1310 ! EFSA-Q-2009-00372 ASB2012-3480	N	---
KIIA 5 KIIIA1 7 (OECD)	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 <i>EFSA Journal</i> 2012;10(11):2986 ASB2012-15513	N	---
KIIA 5 KIIIA1 7 (OECD)	European Commission	2002	Review report for the active substance glyphosate. Finalised in the Standing Committee on Plant Health at its meeting on 29 June 2001 in view of the inclusion of glyphosate in Annex I of Directive 91/414/EEC. Glyphosat 6511/VI/99-final ASB2009-4191	N	---
KIIA 5 KIIIA1 7 (OECD)	Germany	1998	Glyphosate (Monograph) ASB2010-10302	N	---
KIIA 5 KIIIA1 7 (OECD)	Germany	1998	Glyphosate-trimesium (Monograph), ASB2010-10493	N	---
KIIA 5 KIIIA1 7 (OECD)	Germany	2000	Glyphosate (Monograph): Addendum B.6, ASB2013-2748	N	---
KIIA 5 KIIIA1 7 (OECD)	OECD	2002	OECD; Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies ENV/JM/MONO(2002)19 ASB2013-3754	N	---

⁵ Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5 KIIIA1 7 (OECD)	Anonymous	2006	Backgrounder Response to “Glyphosate Toxic & Roundup Worse”. Monsanto statement. http://www.monsanto.com/products/Documents/glyphosate-background-materials/Response_ISIS_apr_06.pdf ASB2013-5455	N	---
KIIA 5.1 K IIA 5.6 (OECD)	Antoniou M, Habib MEEM, Howard CV, Jennings RC, Leifert C, Nodari RO, C Robinson, Fagan J.	2011	Roundup and birth defects: Is the public being kept in the dark? Earth Open Source report. Available from: http://www.earthopensource.org/files/pdfs/Roundup-and-birth-defects/RoundupandBirthDefectsV3.pdf ASB2011-7202	N	---
KIIA 5.1 KIIA 5.10 (OECD)	Carr, K.H., Bleeke, M.S.	2012	Process Description for Identification, Review, and Categorization of Scientific Literature Concerning Glyphosate and AMPA Side-Effects on Health, the Environment, and Non-Target Species k.A. GLP: N, published: Y 2309656 / ASB2012-11583	N	LIT
KIIA 5.1 KIIA 5.10 (OECD)	Klimisch, H.J., Andreae, M., Tillmann, U.	1997	A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data Regulatory Toxicology and Pharmacology 25, 4-5 GLP: N, published: Y 2309856 / ASB2010-14388	N	LIT
KIIA 5.1.1 KIIA 5.5.3 KIIA 5.9 KIIA 5.10 KIIIA1 7.6.4 (OECD)	[REDACTED]	2004	Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study Environmental Health Perspectives 112, 321-326 GLP: N, published: Y 2309536 / ASB2012-11528	N	LIT
KIIA 5.1.1 KIIA 5.4.4 KIIA 5.7.4 KIIA 5.10 (OECD)	[REDACTED]	2009	Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats Toxicol Lett 190, 91-95 GLP: N, published: Y 2309568 / ASB2012-11542	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.1.1 (OECD)	[REDACTED]	1995	Glyphosate: ADME-study in rats - Final report A&M 038/94, TOX9552251	N	---
KIIA 5.1.1 KIIA 5.4.4 (OECD)	[REDACTED]	1991	Metabolism of glyphosate in Sprague-Dawley rats: Tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose, Fundamental and Applied Toxicology 17(1991): 43-51 TOX9551791	N	---
KIIA 5.1.1 KIIA 5.3.2 KIIA 5.4 KIIA 5.5 KIIA 5.10 (OECD)	[REDACTED]	1992	NTP technical report on toxicity studies of Glyphosate administered in dosed feed to F344/N rats and B6C3F1 mice, National Institutes of Health 16(1992) 1-57 TOX9551954	N	---
KIIA 5.1.1 (OECD)	[REDACTED]	1973	Final report on CP 67573 residue and metabolism. Part 9: The gross distribution of n-phosphonomethylglycine- ¹⁴ C in the rabbit TOX9552353	N	---
KIIA 5.1.1 (OECD)	[REDACTED]	1973	CP 67573 residue and metabolism. Part 13: The dynamics of accumulation and depletion of orally ingested N-phosphonomethylglycine- ¹⁴ C TOX9552355	N	---
KIIA 5.1.1 (OECD)	Davies, D.J.	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat CTL/4940 SYN GLP: Y, published: N 2309074 / TOX2000-1977	5.4N	SYN
KIIA 5.1.1 (OECD)	Davies, D.J.	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat CTL/4942 SYN GLP: Y, published: N 2309076 / TOX2000-1978	N	SYN
KIIA 5.1.1 KIIA 5.1.3 (OECD)	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 SYN GLP: Y, published: N 2309078 / TOX2000-1979	N	SYN
KIIA 5.1.1 KIIA 5.1.3 (OECD)	Davies, D. J.	1996	Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg) CTL/P/4943 SYN GLP: Y, published: N 2309080 / TOX2000-1980	N	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.1.1 KIIA 5.9 KIIA 5.10 (OECD)	[REDACTED]	2013	Glyphosate and AMPA: Determination of glyphosate residues in human urine samples from 18 European countries Medical Laboratory Bremen, MLHB-2013-06-06 ASB2013-8037	N	---
KIIA 5.1.1 (OECD)	[REDACTED]	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, TOX9552357	N	---
KIIA 5.1.1 (OECD)	[REDACTED]	1996	[¹⁴ C]-glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat 1413/2-1011 NUF GLP: Y, published: N 2309072 / ASB2012-11380	Y	NUF
KIIA 5.1.1 (OECD)	[REDACTED]	1995	Metabolism study of ¹⁴ C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley rats, 9202/95 TOX9650071	N	---
KIIA 5.1.1 KIIA 5.1.3 (OECD)	[REDACTED]	1996	Glyphosate acid: Biotransformation in the rat CTL/P/5058 SYN GLP: Y, published: N 2309082 / TOX2000-1981	N	SYN
KIIA 5.1.1 KIIA 5.10 (OECD)	Mage, D.T.	2006	Suggested corrections to the Farm Family Exposure Study Environmental Health Perspectives 114, A633-A634 GLP: N, published: Y 2309900 / ASB2012-11888	N	LIT
KIIA 5.1.1 (OECD)	[REDACTED]	1995	HR-001: Metabolism in the rat SNY 332/951256 HLS GLP: Y, published: N 2309070 / ASB2012-11379	N	ALS
KIIA 5.1.1 (OECD)	[REDACTED]	1992	(¹⁴ C)-glyphosate: Absorption and distribution in the rat - preliminary study TOX9552358	N	---
KIIA 5.1.1 (OECD)	[REDACTED]	1992	(¹⁴ C)-glyphosate: Absorption, distribution, metabolism and excretion in the rat, TOX9300343	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.1.1 (OECD)	[REDACTED]	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 TOX9552356	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	2007	Glyphosate technical material: Acute oral toxicity study in rats (Up and Down procedure) B02755; T007035-05 SYN GLP: Y, published: N 2309111 / ASB2012-11391	Y	SYN
KIIA 5.2.1 (OECD)	[REDACTED]	1981	Acute oral toxicity of MON 0139 to rats 800257 ! ML-80-261 TOX9552321	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1990	Acute oral toxicity in the rat: Glyphosate technical R231 ! AGC-900823B ! AGC-101 TOX9500261	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1995	Glyphosate technical 95 %: Acute oral toxicity (LD ₅₀) test in rat 10670 ! IRI 556073 TOX9500377	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1989	Glyphosate technical: Acute oral toxicity (limit) test in rats 5883 ! IRI 243268 TOX9552319	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1996	Assessment of acute oral toxicity of "Glyphosate technical" to mice - incl. Addendum 12321 TOX9552320	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	2008	Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical RF -3996.305.475.07 HAG GLP: Y, published: N 2309100 / ASB2012-11389	Y	HAG
KIIA 5.2.1 (OECD)	[REDACTED]	1996	Glyphosate Acid: Acute Oral Toxicity Study in Rats CTL/P/4660 SYN GLP: Y, published: N 2309109 / TOX2000-1982	N	SYN
KIIA 5.2.1 (OECD)	[REDACTED]	1994	Glyphosate premix: Acute oral toxicity (limit test) in the rat 545/37 TOX9552322	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.1 (OECD)	[REDACTED]	1995	Acute Toxicity Study of MON 0139 By Oral Administration in Mice XX-95-205 MON GLP: Y, published: N 2309115 / ASB2012-11393	Y	MON
KIIA 5.2.1 (OECD)	[REDACTED]	2009	Acute Oral Toxicity Study of Glyphosate TC in Rats 23910 HAG GLP: Y, published: N 2309092 / ASB2012-11385	Y	HAG
KIIA 5.2.1 (OECD)	[REDACTED]	2010	Acute Oral Toxicity Study of Glyphosate TC in Rats 24874 HAG GLP: Y, published: N 2309094 / ASB2012-11386	Y	HAG
KIIA 5.2.1 (OECD)	[REDACTED]	2010	Acute Oral Toxicity Study of Glyphosate TC in Rats 24602 HAG GLP: Y, published: N 2309096 / ASB2012-11387	Y	HAG
KIIA 5.2.1 (OECD)	[REDACTED]	1979	Acute Oral Toxicity Study in Rats. BD-77-428 MON GLP: N, published: N 2309107 / Z35541	N	MON
KIIA 5.2.1 (OECD)	[REDACTED]	1995	HR-001: Acute Oral Toxicity Study In Rats IET 94-0134 ALS GLP: Y, published: N 2309086 / ASB2012-11382	Y	ALS
KIIA 5.2.1 (OECD)	[REDACTED]	1995	HR-001: Acute Oral Toxicity Study In Mice IET 94-0133 ALS GLP: Y, published: N 2309088 / ASB2012-11383	Y	ALS
KIIA 5.2.1 (OECD)	[REDACTED]	2005	Glyphosate Acid Technical - Acute Oral Toxicity Up and Down Procedure in Rats PSL 15274 HAG GLP: Y, published: N 2309098 / ASB2012-11388	Y	HAG
KIIA 5.2.1 (OECD)	[REDACTED]	1999	NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats - Limit test 7907 NUF GLP: Y, published: N 2309117 / ASB2012-11394	Y	NUF
KIIA 5.2.1 (OECD)	[REDACTED]	1987	Acute oral LD ₅₀ study of MON-8750 in Sprague-Dawley rats FDRL 9308A TOX9552323	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.1 (OECD)	[REDACTED]	1987	Acute oral toxicity of MON 8750 in Sprague-Dawley rats FD-86-431/9308A Z85869	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1988	Acute Oral Toxicity Study of Glyphosate Batch/lot/nbr no. XLI-55 in Sprague/Dawley rats FD-88-29 (FDRL 88.20 MON 88.2053.007) GLP: Y, published: N 2309105 / Z35389	N	MON
KIIA 5.2.1 (OECD)	[REDACTED]	1995	Final report for oral and dermal LD ₅₀ tests with Sanachem glyphosate acid technical in rats, limit test 00917 TOX9650909	N	---
KIIA 5.2.1 KIIA 5.2.2 (OECD)	[REDACTED]	1995	Final report for oral and dermal LD ₅₀ tests with Sanachem glyphosate 62 % IPA in rats, limit test 00926 TOX9650910	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	2009	Glyphosate Technical: Acute oral Toxicity Study in Rat, C22864, C22864 EXC GLP: Y, published: N 2309090 / ASB2012-11384	Y	EXC
KIIA 5.2.1 (OECD)	[REDACTED]	1994	Glyphosate: Acute oral toxicity (limit test) in the rat 710/14 TOX9500245	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	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 TOX9551088	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	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 TOX9551089	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	2007	GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study in rats BO2272 NUF GLP: Y, published: N 2309103 / ASB2012-11390	Y	NUF

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.1 (OECD)	[REDACTED]	2011	Glyphosate technical - Acute Oral Toxicity Study in the Rat (Up and Down Procedure) 10/218-001P SYN GLP: Y, published: N 2309113 / ASB2012-11392	Y	SYN
KIIA 5.2.1 (OECD)	[REDACTED]	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute oral toxicity in rats GHA-94-401/R TOX9650142	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1994	Glyphosate technical: Acute oral toxicity study in mice 940020 ! PRO629 TOX9551624	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1989	Acute oral toxicity study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in rats 238050 ! PRO439 TOX9551623	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1992	Glyphosate technical: Acute oral toxicity (limit test) in the rat 134/37 TOX9551810	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1987	Acute oral toxicity of 41 % SN750721 solution in mice - Test report entrusted by Shinung Corporation TX58AO2 TOX9500376	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	1987	Acute oral toxicity of 64 % SN750721 technical liquid in mice Test report entrusted by Shinung Corporation TX58AO1 TOX9500375	N	---
KIIA 5.2.1 (OECD)	[REDACTED]	2009	Glyphosate: Acute Oral Toxicity Study (UDP) In Rats 12170-08 HEL GLP: Y, published: N 2309084 / ASB2012-11381	Y	HAG
KIIA 5.2.2 (OECD)	[REDACTED]	2007	Glyphosate technical material: Acute dermal toxicity study in rats B02766 (T007036-05) SYN GLP: Y, published: N 2309141 / ASB2012-11404	Y	SYN
KIIA 5.2.2 (OECD)	[REDACTED]	1981	Acute dermal toxicity of MON 0139 to rabbits 800258 ! ML-80-261 TOX9552326	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.2 (OECD)	[REDACTED]	1990	Acute dermal toxicity study in the rat: Glyphosate technical AGC-900823A ! AGC-301 ! R232 TOX9551793	N	---
KIIA 5.2.2 (OECD)	[REDACTED]	1987	Acute dermal toxicity study of Mon 8750 in New Zealand white rabbits FDRL 9308A ! FD-86-431 TOX9552327	N	---
KIIA 5.2.2 (OECD)	[REDACTED]	1987	Acute dermal toxicity study of Mon 8722 in New Zealand white rabbits FDRL 9307A ! FD-86-430 TOX9552328	N	---
KIIA 5.2.2 (OECD)	[REDACTED]	1989	Glyphosate Technical Acute Dermal Toxicity (Limit) Test in Rats 5884 CHE GLP: Y, published: N 2309119 / TOX9300328	N	CHE
KIIA 5.2.2 (OECD)	[REDACTED]	2008	Acute Dermal Toxicity in Wistar Hannover Rats for Glyphosate Technical RF-3996.310.456.07 HAG GLP: Y, published: N 2309135 / ASB2012-11402	Y	HAG
KIIA 5.2.2 (OECD)	[REDACTED]	1996	Glyphosate Acid: Acute Dermal Toxicity in the Rat CTL/P/4664 SYN GLP: Y, published: N 2309139 / TOX2000-1983	Y	SYN
KIIA 5.2.2 (OECD)	[REDACTED]	2009	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 23912 HAG GLP: Y, published: N 2309127 / ASB2012-11398	Y	HAG
KIIA 5.2.2 (OECD)	[REDACTED]	2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 24876 HAG GLP: Y, published: N 2309129 / ASB2012-11399	Y	HAG
KIIA 5.2.2 (OECD)	[REDACTED]	2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 24604 HAG GLP: Y, published: N 2309131 / ASB2012-11400	Y	HAG
KIIA 5.2.2 (OECD)	[REDACTED]	1995	HR-001: Acute dermal toxicity study in rats IET 94-0154 ALS GLP: Y, published: N 2309123 / ASB2012-11396	N	ALS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.2 (OECD)	[REDACTED]	2005	Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats - Limit Test PSL 15275 HAG GLP: Y, published: N 2309133 / ASB2012-11401	Y	HAG
KIIA 5.2.2 (OECD)	[REDACTED]	1994	Acute dermal toxicity of glyphosate technical in the rat T1586.3.A TOX9500378	N	---
KIIA 5.2.2 (OECD)	[REDACTED]	1988	Acute dermal toxicity of glyphosate Batch/lot/nbr no. XLI-55 in new zealand white rabbits 88.2053.008 ! FD-88-29 TOX9552325	N	---
KIIA 5.2.2 (OECD)	[REDACTED]	2009	Glyphosate Technical: Acute Dermal Toxicity Study in Rat C22875 EXC GLP: Y, published: N 2309125 / ASB2012-11397	Y	EXC
KIIA 5.2.2 (OECD)	[REDACTED]	1994	Glyphosate: Acute dermal toxicity (limit test) in the rat 710/15 TOX9500246	N	---
KIIA 5.2.2 (OECD)	[REDACTED]	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 TOX9551090	N	---
KIIA 5.2.2 (OECD)	[REDACTED]	2007	GLYPHOSATE TECHNICAL (NUP05068): Acute dermal toxicity study in rats B02283 NUF GLP: Y, published: N 2309137 / ASB2012-11403	Y	NUF
KIIA 5.2.2 (OECD)	[REDACTED]	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute dermal toxicity in rats GHA-94-402/R TOX9650143	N	---
KIIA 5.2.2 (OECD)	[REDACTED]	1989	Acute dermal toxicity study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in rats 238061 ! PRO425 TOX9551625	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.2 (OECD)	[REDACTED]	1992	Glyphosate technical: Acute dermal toxicity (limit test) in the rat 134/38 TOX9551813	N	---
KIIA 5.2.2 (OECD)	[REDACTED]	2009	Glyphosate - Acute Dermal Toxicity Study in Rats 12171-08 HAG GLP: Y, published: N 2309121 / ASB2012-11395	Y	HAG
KIIA 5.2.2 (OECD)	[REDACTED]	2011	Glyphosate Technical - Acute Dermal Toxicity Study in Rats - Final Report Amendment 1 10/218-002P SYN GLP: Y, published: N 2309143 / ASB2012-11405	Y	SYN
KIIA 5.2.3 (OECD)	[REDACTED]	1988	Acute inhalation study of MON 8750 technical EHL 87147 ! ML-87-228 TOX9552332	N	---
KIIA 5.2.3 (OECD)	[REDACTED]	1994	Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat 523-001 ! 545/39 TOX9552331	N	---
KIIA 5.2.3 (OECD)	[REDACTED]	1995	Glyphosate: Acute inhalation toxicity study four-hour exposure (nose only) in the rat 710/16 TOX9500247	N	---
KIIA 5.2.3 (OECD)	[REDACTED]	2004	An acute nose-only inhalation toxicity study in rats with MON 78623 SB-2003-116 MON GLP: Y, published: N 2309169 / ASB2012-11417	Y	MON
KIIA 5.2.3 (OECD)	[REDACTED]	2009	Glyphosate - Acute Inhalation Toxicity Study in Rats 12107-08 HAG GLP: Y, published: N 2309155 / ASB2012-11411	Y	HAG
KIIA 5.2.3 (OECD)	[REDACTED]	2007	Glyphosate technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats B02327 NUF GLP: Y, published: N 2309161 / ASB2012-11414	Y	NUF
KIIA 5.2.3 (OECD)	[REDACTED]	1987	Acute toxicity of Rodeo herbicide administered by inhalation to male and female Sprague-Dawley rats EHL 86105 ! ML-86-281 ! MSL 6582 TOX9552330	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.3 (OECD)	[REDACTED]	2009	Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat 2743/0001 EXC GLP: Y, published: N 2309149 / ASB2012-11408	Y	EXC
KIIA 5.2.3 (OECD)	[REDACTED]	2009	Acute Inhalation Toxicity Study of Glyphosate TC in Rats LPT 23911 HAG GLP: Y, published: N 2309151 / ASB2012-11409	Y	HAG
KIIA 5.2.3 (OECD)	[REDACTED]	2010	Acute Inhalation Toxicity Study of Glyphosate TC In Rats 24603 HEL GLP: Y, published: N 2309145 / ASB2012-11406	Y	HAG
KIIA 5.2.3 (OECD)	[REDACTED]	2010	Acute Inhalation Toxicity Study of Glyphosate TC in Rats LPT 24875 HAG GLP: Y, published: N 2309153 / ASB2012-11410	Y	HAG
KIIA 5.2.3 (OECD)	[REDACTED]	1995	HR-001: Acute inhalation toxicity study in rats IET 94-0155 ALS GLP: Y, published: N 2309147 / ASB2012-11407	Y	ALS
KIIA 5.2.3 (OECD)	[REDACTED]	1989	Glyphosate technical: Acute inhalation toxicity study in rats (limit test) 9993 ! IRI 642062 TOX9552329	N	---
KIIA 5.2.3 (OECD)	[REDACTED]	2005	Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats - Limit Test PSL 15276 HAG GLP: Y, published: N 2309157 / ASB2012-11412	Y	HAG
KIIA 5.2.3 (OECD)	[REDACTED]	2011	Glyphosate Technical - Acute inhalation Toxicity Study (Nose-only) in the Rat 11/054-004P SYN GLP: Y, published: N 2309165 / ASB2012-11415	Y	SYN
KIIA 5.2.3 (OECD)	[REDACTED]	1996	Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study in the Rat CTL/P/4882 SYN GLP: Y, published: N 2309163 / TOX2000-1984	Y	SYN
KIIA 5.2.3 (OECD)	[REDACTED]	1989	4-hour, acute inhalation toxicity study with glyphosate technical in rats 238105 ! PRO426 TOX9551626	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.3 (OECD)	[REDACTED]	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute inhalation toxicity in rats GHA-94-403/R TOX9650144	N	---
KIIA 5.2.3 (OECD)	[REDACTED]	1999	NUP5a99 62 % glyphosate MUP: Acute inhalation toxicity study in rats - Limit test 7909 NUF GLP: Y, published: N 2309167 / ASB2012-11416	Y	NUF
KIIA 5.2.4 (OECD)	[REDACTED]	2007	Glyphosate technical material: Primary skin irritation study in rabbits (4-hour semi-occlusive application) B02777 (T007037-05) SYN GLP: Y, published: N 2309193 / ASB2012-11426	Y	SYN
KIIA 5.2.4 (OECD)	[REDACTED]	1990	Acute dermal irritation/corrosion of glyphosate technical in the rabbit (intact and abraded skin) AGC-900822A ! AGC-001 ! R233 TOX9551794	N	---
KIIA 5.2.4 (OECD)	[REDACTED]	1987	Primary dermal irritation study of Mon-8750 in New Zealand white rabbits FDRL-9308A ! FD-86-431 TOX9552336	N	---
KIIA 5.2.4 (OECD)	[REDACTED]	2008	Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical RF-3996.311.476.07 HAG GLP: Y, published: N 2309185 / ASB2012-11425	Y	HAG
KIIA 5.2.4 (OECD)	[REDACTED]	1989	Glyphosate technical: Primary skin irritation test in rabbits 5885 ! IRI 243268 TOX9552333	N	---
KIIA 5.2.4 (OECD)	[REDACTED]	1996	Glyphosate Acid: Skin Irritation To The Rabbit CTL/P/4695 SYN GLP: Y, published: N 2309191 / TOX2000-1985	Y	SYN
KIIA 5.2.4 (OECD)	[REDACTED]	1994	Glyphosate premix: Acute dermal irritation test in the rabbit 565-003 ! 545/40 TOX9552335	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.4 (OECD)	[REDACTED]	1995	HR-001: Primary Dermal irritation study in rabbits IET 95-0035 ALS GLP: Y, published: N 2309175 / ASB2012-11420	Y	ALS
KIIA 5.2.4 (OECD)	[REDACTED]	2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC In Rabbits 24877 HEL GLP: Y, published: N 2309173 / ASB2012-11419	Y	HAG
KIIA 5.2.4 (OECD)	[REDACTED]	2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits LPT 23913 HAG GLP: Y, published: N 2309177 / ASB2012-11421	Y	HAG
KIIA 5.2.4 (OECD)	[REDACTED]	2010	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits LPT 24605 HAG GLP: Y, published: N 2309179 / ASB2012-11422	Y	HAG
KIIA 5.2.4 (OECD)	[REDACTED]	2005	Glyphosate Acid Technical - Primary Skin Irritation Study in Rabbits PSL 15278 HAG GLP: Y, published: N 2309183 / ASB2012-11424	Y	HAG
KIIA 5.2.4 (OECD)	[REDACTED]	1988	Primary Dermal Irritation Study of Glyphosate Batch/lot/nbr no. XLI-55 in New Zealand White Rabbits FD-88-29 (FDRL 88.20 MON GLP: Y, published: N 2309187 / Z35394	N	MON
KIIA 5.2.4 (OECD)	[REDACTED]	1994	Glyphosate 360g/L: Acute dermal irritation test in the rabbit 710/29 TOX9500248	N	---
KIIA 5.2.4 (OECD)	[REDACTED]	1991	Primary skin irritation study with glyphosate technical (FSG 03090 H/05 march 90) in New Zealand white rabbits ES.878.SKIN ! TOXI-878/1990 ! ES-GPT-SKIN TOX9551092	N	---
KIIA 5.2.4 (OECD)	[REDACTED]	2007	Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) B02294 NUF GLP: Y, published: N 2309171 / ASB2012-11418	Y	NUF

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.4 (OECD)	[REDACTED]	1994	Glyphosate (Alkaloida, Tiszavasvari): Primary dermal irritation study in rabbits GHA-93-404/N TOX9650145	N	---
KIIA 5.2.4 (OECD)	[REDACTED]	1991	Acute dermal irritation study in New Zealand White rabbits treated with the test article glyphosate tecnico 98 % 910259 ! PRO495 TOX9551627	N	---
KIIA 5.2.4 (OECD)	[REDACTED]	1989	Primary skin irritation study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in rabbits (4-hour semi-occlusive application on intact and abraded skin) 238072 ! PRO438 TOX9551628	N	---
KIIA 5.2.4 (OECD)	[REDACTED]	2009	Glyphosate - Acute Dermal Irritation Study in Rabbits 12173-08 HAG GLP: Y, published: N 2309181 / ASB2012-11423	Y	HAG
KIIA 5.2.4 (OECD)	[REDACTED]	2011	Glyphosate technical - Primary skin irritation study in rabbits - Final report Amendment 1 10218-006N SYN GLP: Y, published: N 2309195 / ASB2012-11427	Y	SYN
KIIA 5.2.5 (OECD)	[REDACTED]	2007	Glyphosate technical material: Primary eye irritation study in rabbits B02788 (T007038-05) SYN GLP: Y, published: N 2309219 / ASB2012-11437	Y	SYN
KIIA 5.2.5 (OECD)	[REDACTED]	1990	Acute eye irritation/corrosion of glyphosate technical in the rabbit AGC-900822 ! AGC-002 ! R234 TOX9500264	N	---
KIIA 5.2.5 (OECD)	[REDACTED]	1987	Primary eye irritation of Mon 8722 in New Zealand white rabbits FDRL 9307A ! FD-86-430 TOX9552342	N	---
KIIA 5.2.5 (OECD)	[REDACTED]	2008	Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical RF-3996.312.599.07 HAG GLP: Y, published: N 2309213 / ASB2012-11436	Y	HAG

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.5 (OECD)	[REDACTED]	1989	Glyphosate technical: Primary eye irritation test in rabbits 5886 ! IRI 243268 TOX9552338	N	---
KIIA 5.2.5 (OECD)	[REDACTED]	1994	Glyphosate premix: Acute eye irritation test in the rabbit 566-003 ! 545/41 TOX9552340	N	---
KIIA 5.2.5 (OECD)	[REDACTED]	1995	HR-001: Primary Eye Irritation study in rabbits IET 95-0034 ALS GLP: Y, published: N 2309201 / ASB2012-11430	Y	ALS
KIIA 5.2.5 (OECD)	[REDACTED]	1997	Glyphosate Acid: Eye Irritation to the Rabbit CTL/P/5138 SYN GLP: Y, published: N 2309217 / TOX2000-4986	Y	SYN
KIIA 5.2.5 (OECD)	[REDACTED]	1996	CHA 440: Primary eye irritation study in rabbits 2981-96 ! S9-EE81-4.C41 TOX1999-881	N	---
KIIA 5.2.5 (OECD)	[REDACTED]	2009	Acute Eye Irritation/Corrosion Test Of Glyphosate TC In Rabbits 24878 HEL GLP: Y, published: N 2309199 / ASB2012-11429	Y	HAG
KIIA 5.2.5 (OECD)	[REDACTED]	2009	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits LPT 23914 HAG GLP: Y, published: N 2309205 / ASB2012-11432	Y	HAG
KIIA 5.2.5 (OECD)	[REDACTED]	2010	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits LPT 24606 HAG GLP: Y, published: N 2309207 / ASB2012-11433	Y	HAG
KIIA 5.2.5 (OECD)	[REDACTED]	2005	Eye Irritation/Corrosion Effects in Rabbits (<i>Oryctolagus cuniculus</i>) of Glyphosate 95 TC PSL 15277 HAG GLP: Y, published: N 2309211 / ASB2012-11435	Y	HAG
KIIA 5.2.5 (OECD)	[REDACTED]	1988	Primary Eye Irritation Study of Glyphosate FD-88-29 MON GLP: N, published: N 2309215 / Z35395	N	MON

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.5 (OECD)	[REDACTED]	2009	Expert Statement Expert Statement Expert Statement Glyphosate technical: Primary eye irritation study in rat C22897 EXC GLP: Y, published: N 2309203 / ASB2012-11431	Y	EXC
KIIA 5.2.5 (OECD)	[REDACTED]	1994	Glyphosate: Acute eye irritation test in the rabbit 710/18 TOX9500249	N	---
KIIA 5.2.5 (OECD)	[REDACTED]	1991	Primary eye irritation study with glyphosate technical (FSG 03090 H/05 march 90) in New Zealand white rabbits ES.879.EYE ! TOXI-879/1990 ! ES-GPT- EYE TOX9551093	N	---
KIIA 5.2.5 (OECD)	[REDACTED]	2007	Glyphosate Technical (NUP 05068): Primary Eye Irritation Study In Rabbits B02305 NUF GLP: Y, published: N 2309197 / ASB2012-11428	Y	NUF
KIIA 5.2.5 (OECD)	[REDACTED]	2011	Glyphosate Technical - Acute Eye Irritation Study in Rabbits 10/218-005N NUF GLP: Y, published: N 2309221 / ASB2012-11438	Y	SYN
KIIA 5.2.5 (OECD)	[REDACTED]	1994	Glyphosate (Alkaloida, Tiszavasvari): Primary eye irritation study in rabbits GHA-93-405/N TOX9650146	N	---
KIIA 5.2.5 (OECD)	[REDACTED]	1991	Acute eye irritation study in New Zealand White rabbits treated with the test article glyphosate tecnico 98 % 910260 ! PRO496 Z101610	N	---
KIIA 5.2.5 (OECD)	[REDACTED]	1989	Primary eye irritation with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N- phosphonomethylglycine acid) in the rabbit (rinsed / unrinsed eyes) 238083 ! PRO423 TOX9551629	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.5 (OECD)	[REDACTED]	2009	Glyphosate - Acute Eye Irritation Study in Rabbits 12172-08 HAG GLP: Y, published: N 2309209 / ASB2012-11434	Y	HAG
KIIA 5.2.6 (OECD)	[REDACTED]	1983	A dermal sensitization study in guinea pigs with Glyphosate BD-83-008 ! B/d 4235-82 Z35238	N	---
KIIA 5.2.6 (OECD)	[REDACTED]	2007	Glyphosate Technical Material - Skin Sensitisation (Local Lymph Node Assay in the Mouse) GM8048-REG SYN GLP: Y, published: N 2309245 / ASB2012-11449	Y	SYN
KIIA 5.2.6 (OECD)	[REDACTED]	1989	Glyphosate technical: Magnusson-Kligman maximisation test in guinea pigs 5887 ! IRI 243268 TOX9552343	N	---
KIIA 5.2.6 (OECD)	[REDACTED]	1996	Glyphosate Acid: Skin Sensitisation to the Guinea Pig CTL/P/4699 SYN GLP: Y, published: N 2309243 / TOX2000-1987	Y	SYN
KIIA 5.2.6 (OECD)	[REDACTED]	1994	Glyphosate premix: Magnusson & Kligman maximisation study in the guinea pig 567-003 ! 545/42 TOX9552345	N	---
KIIA 5.2.6 (OECD)	[REDACTED]	2009	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) LPT 23915 HAG GLP: Y, published: N 2309231 / ASB2012-11443	Y	HAG
KIIA 5.2.6 (OECD)	[REDACTED]	2010	Examination Of Glyphosate TC In The Skin Sensitisation Test In Guinea Pigs According To Magnusson And Kligman (Maximisation Test) 24879 HEL GLP: Y, published: N 2309225 / ASB2012-11440	Y	HAG
KIIA 5.2.6 (OECD)	[REDACTED]	2010	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) LPT 24607 HAG GLP: Y, published: N 2309233 / ASB2012-11444	Y	HAG

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.6 (OECD)	[REDACTED]	1995	HR-001: Dermal sensitisation study in guinea pigs IET 95-0036 ALS GLP: Y, published: N 2309227 / ASB2012-11441	Y	ALS
KIIA 5.2.6 (OECD)	[REDACTED]	2008	Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test RF-3996.318.431.07 HAG GLP: Y, published: N 2309239 / ASB2012-11447	Y	HAG
KIIA 5.2.6 (OECD)	[REDACTED]	2005	Glyphosate acid technical - Dermal Sensitisation in Guinea Pigs (Buehler Method) PSL 15279 HAG GLP: Y, published: N 2309237 / ASB2012-11446	Y	HAG
KIIA 5.2.6 (OECD)	[REDACTED]	1993	Skin sensitisation test in guinea-pigs with glyphosate technical 95 % min of Excel Industries Ltd., Bombay. IIT 1230 TOX9650652	N	---
KIIA 5.2.6 (OECD)	[REDACTED]	2006	Glyphosate Technical: Skin Sensitisation in the Guinea Pig - Magnusson and Kligman Maximisation method 2060/009 (SMK-PH-05- NUF GLP: Y, published: N 2309241 / ASB2012-11448	Y	NUF
KIIA 5.2.6 (OECD)	[REDACTED]	2009	Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs - Maximisation-Test C22908 EXC GLP: Y, published: N 2309229 / ASB2012-11442	Y	EXC
KIIA 5.2.6 (OECD)	[REDACTED]	1994	Glyphosate: Magnusson & Kligman maximisation study in the guinea pig 710/19 TOX9500250	N	---
KIIA 5.2.6 (OECD)	[REDACTED]	2007	Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test B02316 NUF GLP: Y, published: N 2309223 / ASB2012-11439	Y	NUF
KIIA 5.2.6 (OECD)	[REDACTED]	2011	Glyphosate technical - Local lymph node assay in the mouse - Final report amendment 2 10/218-037E SYN GLP: Y, published: N 2309247 / ASB2012-11450	Y	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.2.6 (OECD)	[REDACTED]	1991	Luxan glyphosate techn.: Magnusson & Kligman maximisation study in the guinea pig 349/11 TOX9551796	N	---
KIIA 5.2.6 (OECD)	[REDACTED]	2009	Glyphosate - Skin Sensitisation Study in Guinea Pigs. Buehler Test 12174-08 HAG GLP: Y, published: N 2309235 / ASB2012-11445	Y	HAG
KIIA 5.3.1 (OECD)	[REDACTED]	1989	Glyphosate: 4 week dietary toxicity study in rats 5626 ! IRI 437462 TOX9552351	N	---
KIIA 5.3.1 (OECD)	[REDACTED]	1989	Glyphosate: Oral maximum tolerated dose study in dogs 5660 ! IRI 640683 TOX9552352	N	---
KIIA 5.3.1 KIIIA1 7.6.2 (OECD)	[REDACTED]	2012	Glyphosate acid - <i>In Vitro</i> absorption through abraded rabbit skin using [¹⁴ C]-glyphosate JV2182-REG GTF GLP: Y, published: N 2309282 / ASB2012-11459	Y	EGT
KIIA 5.3.1 (OECD)	[REDACTED]	1993	Glyphosate: 3 week toxicity study in rats with dermal administration 7839 ! IRI 450881 TOX9552367	N	---
KIIA 5.3.1 KIIA 5.3.7 KIIIA1 7.6.2 (OECD)	[REDACTED]	1982	21-Day dermal toxicity study in rabbits IR-81-195 MON GLP: N, published: N 2309280 / TOX9552366	N	MON
KIIA 5.3.1 (OECD)	[REDACTED]	1982	Range finding study of MON 0139 and isopropylamine administered orally to dogs ML-81-032/810036 ! MSL-2155 TOX9552349	N	---
KIIA 5.3.1 (OECD)	[REDACTED]	1996	Glyphosate acid: 21-day dermal toxicity study in rats CTL/P/4985 SYN GLP: Y, published: N 2309288 / ASB2012-11461	Y	SYN
KIIA 5.3.1 (OECD)	[REDACTED]	1991	28-day dietary study in rats on glyphosate technical ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR TOX9551095	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.3.1 (OECD)	[REDACTED]	1994	28-day dietary study in rats on glyphosate technical –Amendment ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR Z102035	N	---
KIIA 5.3.1 (OECD)	[REDACTED]	1994	28-day dietary study in rats on glyphosate technical - Second Amendment ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR Z102043	N	---
KIIA 5.3.1 KIIA 5.3.7 (OECD)	[REDACTED]	1994	GGlyphosate technical (Alkaloida, Tiszavasvári): Repeated dose twenty-eight-Day dermal toxicity study in rabbits MÜF 214/94 MON GLP: Y, published: N 2309284 / TOX9650151	N	MON
KIIA 5.3.1 KIIIA1 7.1.3 (OECD)	[REDACTED]	1983	Four-week study of 33-1/3 % use-dilution of Roundup in water administered to male and female Sprague-Dawley rats by inhalation 830025 ! ML-83-015 TOX2002-694	N	---
KIIA 5.3.2 (OECD)	[REDACTED]	1981	Glyphosate: Subchronic toxicological study 90-day rats TOX9650152	N	---
KIIA 5.3.2 (OECD)	[REDACTED]	1996	First Revision to Glyphosate Acid: 90 Day Oral Feeding Study in Rats CTL/P/1599 SYN GLP: Y, published: N 2309249 / TOX2000-1990	Y	SYN
KIIA 5.3.2 (OECD)	[REDACTED]	1990	Glyphosate technical: 90 day oral toxicity study in the rat AGC-900914 ! AGC-401 ! R230 TOX9500266	N	---
KIIA 5.3.2 (OECD)	[REDACTED]	1996	Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study In The Rat 434/016 NUF GLP: Y, published: N 2309256 / ASB2012-11451	Y	NUF
KIIA 5.3.2 (OECD)	[REDACTED]	1989	Glyphosate technical: 90 day oral toxicity study in the rats - incl. Amendment to Protocol BY-401 BY-891002 ! BY-401 TOX9551821	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.3.2 (OECD)	[REDACTED]	1995	HR-001: 13-week Subchronic Oral Toxicity Study in Rats IET 94-0138 ALS GLP: Y, published: N 2309258 / ASB2012-11452	Y	ALS
KIIA 5.3.2 (OECD)	[REDACTED]	1995	HR-001: 13-week Oral Subchronic Toxicity Study in Mice IET 94-0136 ALS GLP: Y, published: N 2309260 / ASB2012-11453	N	ALS
KIIA 5.3.2 (OECD)	[REDACTED]	1993	90 day range finding study of glyphosate in rats 011-0001 ALK GLP: Y, published: N 2309252 / TOX9650149	N	ALK
KIIA 5.3.2 (OECD)	[REDACTED]	1991	Glyphosate: 13-week dietary toxicity study in rats 7136 ! IRI 437876 TOX9552364	N	---
KIIA 5.3.2 (OECD)	[REDACTED]	1991	Glyphosate: 13-week dietary toxicity study in mice 7024 ! IRI 437918 TOX9552363	N	---
KIIA 5.3.2 (OECD)	[REDACTED]	1987	90-day study of glyphosate administered in feed to Sprague-Dawley rats MSL 7375 ! ML-86-351 ! EHL 86128 TOX9552362	N	---
KIIA 5.3.2 (OECD)	[REDACTED]	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 TOX9551096	N	---
KIIA 5.3.3 (OECD)	[REDACTED]	2007	Glyphosate Technical: 13-Week Toxicity Study By Oral Route (Capsule) In Beagle Dogs 29646 TCC NUF GLP: Y, published: N 2309262 / ASB2012-11454	Y	NUF
KIIA 5.3.3 (OECD)	[REDACTED]	1996	First Revision to Glyphosate Acid: 90-Day Oral Toxicity Study in Dogs CTL/P/1802 SYN GLP: Y, published: N 2309271 / TOX2000-1991	Y	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.3.3 (OECD)	[REDACTED]	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 FSG GLP: Y, published: N 2309264 / ASB2012-11455	Y	FSG
KIIA 5.3.3 (OECD)	[REDACTED]	1985	Twelve month study of glyphosate administered by gelatin capsule to beagle dogs MSL-5069 ! 636 Z35385	N	---
KIIA 5.3.3 (OECD)	[REDACTED]	1983	Six month study of MON 0139 administered by gelatin capsule to beagle dogs 810166 ! ML-81-368 TOX9552361	N	---
KIIA 5.3.3 (OECD)	[REDACTED]	1996	HR-001: 13-week Oral Subchronic Toxicity Study in Dogs IET 94-0158 ALS GLP: Y, published: N 2309269 / ASB2012-11456	Y	ALS
KIIA 5.3.4 (OECD)	[REDACTED]	1996	Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs CTL/P/5079 SYN GLP: Y, published: N 2309278 / TOX2000-1992	Y	SYN
KIIA 5.3.4 (OECD)	[REDACTED]	1990	Glyphosate: 52-week oral toxicity study in dogs 7502 ! IRI 642675 TOX9552384	N	---
KIIA 5.3.4 (OECD)	[REDACTED]	2007	Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs 29647 TCC NUF GLP: Y, published: N 2309274 / ASB2012-11457	Y	NUF
KIIA 5.3.4 (OECD)	[REDACTED]	1997	HR-001: 12-Month Oral Chronic Toxicity Study in Dogs IET 94-0157 ALS GLP: Y, published: N 2309276 / ASB2012-11458	Y	ALS
KIIA 5.4.1 (OECD)	[REDACTED]	1995	HR-001: Reverse Mutation Test IET 94-0142 ALS GLP: Y, published: N 2309291 / ASB2012-11462	Y	ALS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.1 (OECD)	Callander, R.D.	1996	Glyphosate acid: An evaluation of mutagenic potential using <i>S. typhimurium</i> and <i>E. coli</i> CTL/P/4874 SYN GLP: Y, published: N 2309313 / ASB2012-11473	Y	SYN
KIIA 5.4.1 (OECD)	Flügge, C.	2009	Mutagenicity Study of Glyphosate TC in the <i>Salmonella typhimurium</i> Reverse Mutation Assay (<i>in vitro</i>) LPT 23916 HAG GLP: Y, published: N 2309303 / ASB2012-11468	Y	HAG
KIIA 5.4.1 (OECD)	Flügge, C.	2010	Mutagenicity Study of Glyphosate TC in the <i>Salmonella typhimurium</i> Reverse Mutation Assay (<i>in vitro</i>) LPT 24880 HAG GLP: Y, published: N 2309305 / ASB2012-11469	Y	HAG
KIIA 5.4.1 (OECD)	Jensen, J. C.	1991	Mutagenicity test: Ames salmonella assay with glyphosate, batch 206-JaK-25-1 Report: 12323 TOX9552371	Y	---
KIIA 5.4.1 KIIIA1 7.6.3 (OECD)	Kier, L. D.; Stegeman, S. D.; Costello, J. G.; Schermes, S.	1992	Ames/salmonella mutagenicity assay of MON 2139 (Roundup herbicide formulation) EHL 91183 ! ML-91-440 ! MSL-11729 TOX1999-239	N	---
KIIA 5.4.1 (OECD)	Kier, L. D.; Stegeman, S. D.; Costello, J. G.; Schermes, S.	1992	Ames/salmonella mutagenicity assay of MON 14445 (DIRECT Herbicide formulation) MSL-11731 ! EHL 91185/ML-91-442 TOX1999-320	N	---
KIIA 5.4.1 KIIA 5.4.4 (OECD)	Kier, L. D.; Stegeman, S. D.; Costello, J. G.; Schermes, S.	1992	Ames/Salmonella mutagenicity assay of Rodeo MSL-11730 ! EHL 91184/ML-91-441 TOX9552373	N	---
KIIA 5.4.1 (OECD)	Li, A. P.; Long, T. J.	1988	An evaluation of the genotoxic potential of glyphosate, Fundamental and Applied Toxicology 10 (1988)537 – 546 published: Y, TOX9500253	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.1 KIIA 5.4.4 (OECD)	Rank, J.; Jensen, A. G.; Skov, B.	1993	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 Z82234	Y	---
KIIA 5.4.1 KIIA 5.4.4 (OECD)	Rasmussen, E. S.	1997	Genotoxicity of Roundup/Glyphosate, Danish Environmental Protection Agency, AA036753, 7042-0110 ASB2013-9671	N	---
KIIA 5.4.1 (OECD)	Riberri do Val, R.	2007	Bacterial reverse mutation test (Ames Test) for Glifosato Técnico Helm 3393/2007-2.0AM-B HAG GLP: Y, published: N 2309299 / ASB2012-11466	Y	HAG
KIIA 5.4.1 (OECD)	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 Report: ET-78-241, TOX9552368	N	---
KIIA 5.4.1 (OECD)	Sokolowski, A.	2007	<i>Salmonella typhimurium</i> and <i>Escherichia coli</i> Reverse mutation assay with glyphosate technical (NUP-05068) 1061401 NUF GLP: Y, published: N 2309293 / ASB2012-11463	Y	NUF
KIIA 5.4.1 (OECD)	Sokolowski, A.	2007	<i>Salmonella typhimurium</i> and <i>Escherichia coli</i> Reverse mutation assay with glyphosate technical (NUP-05070) 1061402 NUF GLP: Y, published: N 2309295 / ASB2012-11464	Y	NUF
KIIA 5.4.1 (OECD)	Sokolowski, A.	2007	<i>Salmonella typhimurium</i> and <i>Escherichia coli</i> Reverse mutation assay with glyphosate technical (NUP-05067) 1061403 NUF GLP: Y, published: N 2309297 / ASB2012-11465	Y	NUF
KIIA 5.4.1 (OECD)	Sokolowski, A.	2009	Glyphosate technical - <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> Reverse Mutation Assay 1264500 SYN GLP: Y, published: N 2309315 / ASB2012-11474	Y	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.1 (OECD)	Sokolowski, A.	2010	<i>Salmonella typhimurium</i> and <i>Escherichia coli</i> Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine 1332300 HAG GLP: Y, published: N 2309307 / ASB2012-11470	Y	HAG
KIIA 5.4.1 (OECD)	Thompson, P.W.	1996	Technical glyphosate: Reverse mutation assay "Ames test" using <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> 434/014 NUF GLP: Y, published: N 2309311 / ASB2012-11472	Y	NUF
KIIA 5.4.1 (OECD)	Vargas, A. A. T.; Bonetti, R.	1996	The <i>Salmonella typhimurium</i> reverse mutation by Glifos G.1.1 - 050/96 TOX1999-884	N	---
KIIA 5.4.1 (OECD)	Wallner, B.	2010	Reverse Mutation Assay using Bacteria (<i>Salmonella typhimurium</i>) with Glyphosate TC BSL 101268 HAG GLP: Y, published: N 2309309 / ASB2012-11471	Y	HAG
KIIA 5.4.2 (OECD)	Fox, V.	1998	Glyphosate acid: <i>In vitro</i> cytogenetic assay in human lymphocytes CTL/P/6050 SYN GLP: Y, published: N 2309321 / TOX2000-1995	Y	SYN
KIIA 5.4.2 (OECD)	Jensen, J. C.	1991	Mutagenicity test: <i>In vitro</i> mammalian cell gene mutation test with glyphosate, batch 206- JaK-25-1, Report: 12325, published: N, TOX9552372	N	---
KIIA 5.4.2 (OECD)	Kyomu, M.	1995	HR-001: <i>In vitro</i> cytogenetics test IET 94-0143 ALS GLP: Y, published: N 2309317 / ASB2012-11475	Y	ALS
KIIA 5.4.2 KIIA 5.4.3 KIIA 4.4.4 (OECD)	Li, A. P.	1983	CHO/HGPRT gene mutation assay with glyphosate, Report ML-83-155 ! 830079, published: N, TOX9552369	N	---
KIIA 5.4.2 (OECD)	Rossberger, S.	1994	Glyphosate: DNA repair test with primary rat hepatocytes, Report: 931564 ! 94-03-28 ro, published: N, TOX9400697/ TOX9551099	N	FSG
KIIA 5.4.2 (OECD)	van de Waart, E. J.	1995	Evaluation of the ability of glyphosate to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) Report: 141918, published: N, TOX9651525	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.2 (OECD)	Wright, N.P.	1996	Technical glyphosate: Chromosome aberration test in CHL cells <i>in vitro</i> 434/015 NUF GLP: Y, published: N 2309319 / ASB2012-11476	Y	NUF
KIIA 5.4.3 (OECD)	Akanuma, M.	1995	HR-001: DNA Repair Test (Rec-Assay) IET 94-0141 ALS GLP: Y, published: N 2309325 / ASB2012-11477	N	ALS
KIIA 5.4.3 (OECD)	Clay, P.	1996	Glyphosate acid: L5178 TK+/- mouse lymphoma gene mutation assay CTL/P/4991 SYN GLP: Y, published: N 2309323 / TOX2000-1994	Y	SYN
KIIA 5.4.4 KIIA 5.10 (OECD)	Alvarez-Moya, C., Silva, M.R., Arambula, A.R.V., Sandoval, A.I., Vasquez, H.C., Montes, R.M.G.	2011	Evaluation of genetic damage induced by glyphosate isopropylamine salt using Tradescantia bioassays Genetics and Molecular Biology 34 (1):127-130 GLP: N, published: Y 2309560 / ASB2012-11538	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Amer, S.M., Aly, F.A.E., Farghaly, A.A., Ibrahim, A.A.E.	2006	<i>In vitro</i> and <i>in vivo</i> evaluation of the genotoxicity of the herbicide glyphosate in mice Bulletin of the National Research Centre (Egypt) 31, 427-446 GLP: N, published: Y 2309562 / ASB2012-11539	N	MOD
KIIA 5.4.4 KIIA 5.10 (OECD)	Andre, V., Goff, J.L., Pottier, D., Lebailly, P., Peluso, M., Munnia, A., Gauduchon, P.	2007	Evaluation of bulky DNA adduct levels after pesticide use: Comparison between open-field farmers and fruit growers Toxicological & Environmental Chemistry 89, 125-139 GLP: N, published: Y 2309570 / ASB2012-11543	N	LIT
KIIA 5.4.4 KIIA 5.5.3 KIIA 5.6 KIIA 5.7.2 KIIA 5.10 KIIA 5.6.3 (OECD)	Anonym.	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	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.4 KIIA 5.10 (OECD)	Benachour, N., Seralini, G.E.	2009	Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells Chem Res Toxicol 22, 97-105 GLP: N, published: Y 2309606 / ASB2012-11561	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Bolognesi, C., Bonatti, S., Degan, P., Gallerani, E., Peluso, M., Rabboni, R., Roggieri, P., Abbondandolo, A.	1997	Genotoxic activity of glyphosate and its technical formulation roundup Journal of Agricultural and Food Chemistry 45, 1957-1962 GLP: N, published: Y 2309628 / Z59299	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Bolognesi, C., Carrasquilla, G., Volpi, S., Solomon, K.R., Marshall, E.J.	2009	Biomonitoring of genotoxic risk in agricultural workers from five colombian regions: association to occupational exposure to glyphosate J Toxicol Environ Health A 72, 986-997 GLP: N, published: Y 2309630 / ASB2012-11570	N	JCC
KIIA 5.4.4 KIIA 5.10 (OECD)	Bolognesi, C., Landini, E., Perrone, E., Roggieri, P.	2004	Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centromeric probe Mutation Research-Genetic Toxicology and Environmental Mutagenesis 557, 109-117 GLP: N, published: Y 2309634 / ASB2012-11572	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Bolognesi, C., Perrone, E., Landini, E.	2002	Micronucleus monitoring of a floriculturist population from western Liguria, Italy Mutagenesis 175, 391-397 GLP: N, published: Y 2309636 / ASB2012-11573	N	LIT
KIIA 5.4.4 (OECD)		1999	A micronucleus study in mice for glifosate técnico Nufarm RF-G12.79/99 NUF GLP: Y, published: N 2309335 / ASB2012-11482	Y	NUF
KIIA 5.4.4 KIIA 5.10 KIIA 1 7.6.3 (OECD)	Cavalcante, D.G.S.M., Martinez, C.B.R., Sofia, S.H.	2008	Genotoxic effects of Roundup (R) on the fish <i>Prochilodus lineatus</i> Mutation Research-Genetic Toxicology and Environmental Mutagenesis 655, 41-46 GLP: N, published: Y 2309662 / ASB2012-11586	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.4 KIIA 5.10 (OECD)	Cavas, T., Konen, S.	2007	Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (<i>Carassius auratus</i>) exposed to a glyphosate formulation using the micronucleus test and the comet assay Mutagenesis 22, 263-268 GLP: N, published: Y 2309664 / ASB2012-11587	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	[REDACTED]	2000	Glyphosate: Evaluation of chronic activity and possible far -reaching effects - Part 2. Studies on mutagenic activity Pestycydy, 2000, (3-4), 21-25 ASB2013-9830	N	---
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Clements, C.; Ralph, S.; Petrus, M.	1997	Glyphosate: Genotoxicity of select herbicides in <i>Rana catesbeiana</i> tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay Environ. Molec. Mutagen., 29, 277-288 Z101728	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	[REDACTED]	2000	Comparative analysis between micronuclei tests in mice and in peripheral erythrocytes of <i>Oreochromis niloticus</i> in evaluation of mutagenic potential of the agrotoxins deltamethrin, dicofol, glyphosate, and Imazapyr ASB2013-11477	N	---
KIIA 5.4.4 KIIA 5.5.3 KIIA 5.10 (OECD)	[REDACTED]	2000	Glyphosate: Evaluation of chronic activity and possible far - reaching effects - Part 1. Studies on chronic toxicity Pestycydy, 2000, (3 -4), 11-20 ASB2013-9829	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	[REDACTED]	2000	Glyphosate: Evaluation of chronic activity and possible far - reaching effects - Part 3. Prenatal toxicity Pestycydy, 2000, (3-4), 27-31 ASB2013-9831	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Dimitrov, B.D., Gadeva, P.G., Benova, D.K., Bineva, M.V.	2006	Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems Mutagenesis 21, 375-382 GLP: N, published: Y 2309708 / ASB2012-11607	N	LIT
KIIA 5.4.4 (OECD)	[REDACTED]	2006	Glyphosate Technical: Micronucleus Test In The Mouse 2060/014 NUF GLP: Y, published: N 2309327 / ASB2012-11478	Y	NUF

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.4 (OECD)	Flowers, L. J.	1981	Ames/salmonella mutagenicity assay of MON 8080 MSL 1538 ! ML-80-294/800281 TOX1999-319	N	---
KIIA 5.4.4 (OECD)	[REDACTED]	2009	Micronucleus Test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration LPT 23917 HAG GLP: Y, published: N 2309329 / ASB2012-11479	Y	HAG
KIIA 5.4.4 (OECD)	[REDACTED]	1996	Glyphosate acid: mouse bone marrow micronucleus test CTL/P/4954 SYN GLP: Y, published: N 2309337 / TOX2000-1996	N	SYN
KIIA 5.4.4 KIIA 5.10 (OECD)	[REDACTED]	2002	A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides Mutation Research-Genetic Toxicology and Environmental Mutagenesis 518, 145-150 GLP: N, published: Y 2309776 / ASB2012-11834	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Guilherme, S., Gaivão, I., Santos, M.A., Pacheco, M.	2010	European eel (<i>Anguilla anguilla</i>) genotoxic and pro-oxidant responses following short-term exposure to Roundup® a glyphosate-based herbicide Mutagenesis 25, 523-530 GLP: N, published: Y 2309780 / ASB2012-11836	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Helal, A.D., Moussa, H.M.	2005	Chromosomal aberrations induced by glyphosate isopropylamine herbicide and trials for diminuting its toxicity using some chemical inactivators and antioxidant Veterinary Medical Journal Giza 53, 169-187 GLP: N, published: Y 2309794 / ASB2012-11841	N	LIT
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Heydens, W.F., Healy, C.E., Hotz, K.J., Kier, L.D., Martens, M.A., Wilson, A.G.E, Farmer, D.R.	2008	Genotoxic potential of glyphosate formulations: Mode-of-action investigations Journal of Agricultural and Food Chemistry 56, 1517-1523 GLP: N, published: Y 2309802 / ASB2012-11845	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.4 KIIA 5.10 (OECD)	Holeckova, B.	2006	Evaluation of the <i>in vitro</i> effect of glyphosate-based herbicide on bovine lymphocytes using chromosome painting Bulletin of the Veterinary Research Institute in Pulawy 50, 533-536 GLP: N, published: Y 2309806 / ASB2012-11847	N	LIT
KIIA 5.4.4 (OECD)	[REDACTED]	2008	Glyphosate Technical - Micronucleus Assay in Bone Marrow Cells of the Mouse 1158500 SYN GLP: Y, published: N 2309339 / ASB2012-11483	Y	SYN
KIIA 5.4.4 (OECD)	[REDACTED]	1991	Mutagenicity test: Micronucleus test with glyphosate, batch 206-JaK-25-1, Report: 12324, published: N, TOX9552374	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Kale, P.G., Petty, B.T., Walker, S., Ford, J.B., Dehkordi, N., Tarasia, S., Tasie, B.O., Kale, R., Sohni, Y.R.	1995	Mutagenicity testing of 9 herbicides and pesticides currently used in agriculture Environmental and Molecular Mutagenesis 25, 148-153 GLP: N, published: Y 2309834 / 273986, ASB2012-11860	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Kaya, B.: Creus, A.; Yanikoglu, A.; et al.;	2000	Use of the Drosophila wing spot test in the genotoxicity testing of different herbicides ASB2013-9832	N	---
KIIA 5.4.4 (OECD)	[REDACTED]	1992	Mouse micronucleus study of Rodeo herbicide formulation MSL-11772 ! EHL 91201/91205/ML-91-438 TOX9552376	N	---
KIIA 5.4.4 KIIA 1 7.6.3 (OECD)	[REDACTED]	1992	Mouse micronucleus study of Roundup herbicide formulation MSL-11771 ! EHL 91200/91204 ! ML-91-434/ML-91-437 TOX1999-242	N	---
KIIA 5.4.4 (OECD)	[REDACTED]	1992	Glyphosate: Mouse micronucleus study of DIRECT Herbicide formulation MSL-11773 ! EHL 91202/91206 ! ML-91-436/ML-91-439 TOX1999-322	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.4 KIIA 5.10 (OECD)	Knopper, L.D., Lean, D.R.S.	2004	Carcinogenic and genotoxic potential of turf pesticides commonly used on golf courses Journal of Toxicology and Environmental Health-Part B-Critical Reviews 7, 267-279 GLP: N, published: Y 2309864 / ASB2012-11871	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Lebailly, P., Devaux, A., Pottier, D., De Meo, M., Andre, V., Baldi, I., Severin, F., Bernaud, J., Durand, B., Henry-Amar, M., Gauduchon, P.	2003	Urine mutagenicity and lymphocyte DNA damage in fruit growers occupationally exposed to the fungicide captan Occupational & Environmental Medicine 60, 910-917 GLP: N, published: Y 2309878 / ASB2012-11878	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Levine, S.L., Han, Z., Liu, J., Farmer, D.R., Papadopoulos, V.	2007	Disrupting mitochondrial function with surfactants inhibits MA-10 Leydig cell steroidogenesis Cell Biol Toxicol 23, 385-400 GLP: N, published: Y 2309890 / ASB2009-9030	N	LIT
KIIA 5.4.4 (OECD)		1983	<i>In vivo</i> bone marrow cytogenetics study of glyphosate in Sprague-Dawley rats, Report: ML-83-236 ! 830083, published: N, TOX9552375	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)		1998	Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures <i>in vitro</i> Mutation Research 403 (1998) 13-20 ASB2013-9836	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)		1998	Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed <i>in vitro</i> to glyphosate, vinclozolin, atrazine and DPX-E9636 Environmental and Molecular Mutagenesis 32: 39-46 (1998) ASB2013-9837	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.4 KIIA 5.10 (OECD)	Manas, F., Peralta, L., Raviolo, J., Ovandoa, H.G., Weyers, A., Ugnia, L., Cid, M.G., Larripa, I., Gorla, N.	2009	Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests Environmental Toxicology and Pharmacology 28, 37-41 GLP: N, published: Y 2309908 / ASB2012-11892	N	LIT
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)		1991	Glyphosate: Oral and pulmonary toxicology of the surfactant used in Roundup herbicide Z80636	N	---
KIIA 5.4.4 KIIA 5.11 (OECD)		1994	Environmental health criteria 159, Glyphosate TOX9500301	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Mladinic, M., Berend, S., Vrdoljak, A.L., Kopjar, N., Radic, B., Zeljetic, D.	2009	Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro Environmental and Molecular Mutagenesis 50, 800-807 GLP: N, published: Y 2309942 / ASB2012-11906	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Mladinic, M., Perkovic, P., Zeljetic, D.	2009	Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbendazim using cytome FISH assay Toxicol Lett 189, 130-137 GLP: N, published: Y 2309944 / ASB2012-11907	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Monroy, C., Cortes, A., Sicard, D., de Restrepo, H.	2005	Cytotoxicity and genotoxicity of human cells exposed <i>in vitro</i> to glyphosate Biomedica 25, 335-345 GLP: N, published: Y 2309950 / ASB2012-11910	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Pastor, S., Crens, A., Parron, T., Cebulska-Wasilewska, A., Siffel, C., Piperakis, S., Marcos, R.	2003	Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers Mutagenesis 18, 249-258 GLP: N, published: Y 2310004 / ASB2012-11991	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.4 KIIA 5.10 (OECD)	Paz-Y-Mino, C., Sanchez, M.E., Arevalo, M., Munoz, M.J., Witte, T., De-La-Carrera, G.O., Leone, P.E.	2007	Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate Genetics and Molecular Biology 30, 456-460 GLP: N, published: Y 2310006 / ASB2012-11992	N	LIT
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Peluso, M., Munnia, A., Bolognesi, C., Parodi, S.	1998	32P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup Environmental and Molecular Mutagenesis 31, 55-59 GLP: N, published: Y 2310014 / TOX1999-318	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Piesova, E.	2004	The Influence Of Different Treatment Length On the Induction Of Micronuclei In Bovine Lymphocytes After Exposure To Glyphosate Folia Veterinaria 48, 130-134 GLP: N, published: Y 2310026 / ASB2012-12001	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Piesova, E.	2005	The effect of glyphosate on the frequency of micronuclei in bovine lymphocytes <i>in vitro</i> Acta Veterinaria-Beograd 55, 101-109 GLP: N, published: Y 2310024 / ASB2012-12000	N	MOD
KIIA 5.4.4 KIIA 5.10 (OECD)	Poletta, G.L., Larriera, A., Kleinsorge, E., Mudry, M.D.	2009	Genotoxicity of the herbicide formulation Roundup (R) (glyphosate) in broad-snouted caiman (<i>Caiman latirostris</i>) evidenced by the Comet assay and the Micronucleus test Mutation Research-Genetic Toxicology and Environmental Mutagenesis 672, 95-102 GLP: N, published: Y 2310028 / ASB2012-12002	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Prasad, S., Srivastava, S., Singh, M., Shukla, Y.	2009	Clastogenic effects of glyphosate in bone marrow cells of swiss albino mice J Toxicol GLP: N, published: Y 2310034 / ASB2012-12005	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Raipulis, J., Toma, M., Balode, M.	2009	Toxicity and genotoxicity testing of Roundup Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences. 63, 29-32 GLP: N, published: Y 2310040 / ASB2012-12008	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.4 KIIA 5.10 (OECD)	Rodrigues, H.G., Penha-Silva, N., Araujo, M.F.P, Nishijo, H., Aversi-Ferreira, T.A.	2011	Effects of Roundup Pesticide on the Stability of Human Erythrocyte Membranes and Micronuclei Frequency in Bone Marrow Cells of Swiss Mice Open Biology Journal 54-59 GLP: N, published: Y 2310046 / ASB2012-12010	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Salvagni, J., Ternus, R., Fuentefria, A.	2011	Assessment of the genotoxic impact of pesticides on farming communities in the countryside of Santa Catarina State, Brazil Genetics and Molecular Biology 34: 122-126 GLP: N, published: Y 2310060 / ASB2012-12017	N	LIT
KIIA 5.4.4 KIIA 5.9 KIIIA1 7.6.3 (OECD)	Sawada, Y., Nagai, Y.	1987	Roundup® poisoning - its clinical observation possible involvement - englische Version Journal of Clinical and Experimental Medicine (paper) 143: 25-27 GLP: N, published: Y 2309502 / Z35536	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Shaham, J., Kaufman, Z., Gurvich, R., Levi, Z.	2001	Frequency of sister-chromatid exchange among greenhouse farmers exposed to pesticides Mutat Res 491-, 71-80 GLP: N, published: Y 2310076 / ASB2012-12025	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Sivikova, K., Dianovsky, J.	2006	Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes Int J Hyg Environ Health 209, 15-20 GLP: N, published: Y 2310084 / ASB2012-12029	N	LIT
KIIA 5.4.4 KIIA 5.8.1 (OECD)	[REDACTED]	1992	Dodigen 4022: Chromosome aberrations in vitro in V79 chinese hamster cells 92.1024 ! 92.0337 TOX1999-325	N	---
KIIA 5.4.4 KIIA 5.8.1 (OECD)	Stammlberger, I.; Mayer, D.	1992	Dodigen 4022: Study of the mutagenic potential in strains of <i>Salmonella typhimurium</i> (ames test) and <i>Escherichia coli</i> 92.0487 ! 92.0336 TOX1999-324	N	---
KIIA 5.4.4 KIIA 5.10 KIIIA1 7.6.3 (OECD)	[REDACTED]	1998	Mouse micronucleus screening assay of MON 0818 ML-89-463 ! EHL 89182 TOX1999-240	N	---
KIIA 5.4.4 KIIIA1 7.6.3 (OECD)	Stegeman, S. D.; Li, A. P.	1990	Ames/salmonella mutagenicity assay of MON 0818 EHL 89178 ! ML-89-461 ! MSL-10625 TOX1999-241	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.4.4 (OECD)	[REDACTED]	1993	Glyphosate technical (FSG 03090 H/05 March 1990): Mutagenicity-micronucleus test in swiss albino mice, Report: 889-MUT.MN ! TOXI-889/1993 ! ES-GPT-MUT-MN, published: N, TOX9551100	N	---
KIIA 5.4.4 (OECD)	[REDACTED]	1994	Glyphosate technical (FSG 03090 H/05 March 1990): Genetic toxicology - <i>In vivo</i> mammalian bone marrow cytogenetic test, Report: 890-MUT-CH.AB ! TOXI-890/1993 ! ES-GPT-MUT-CH.AB, published: N, TOX9400323 / TOX9551101	N	---
KIIA 5.4.4 KIIA 5.10 (OECD)	Vigfusson, N.V., Vyse, E.R.	1980	The effect of the pesticides Dexton, Captan and Roundup on sister chromatid exchanges in human lymphocytes <i>in vitro</i> , Mutation Research 79, 53-57 GLP: N, published: Y 2310114 / TOX970056 / ASB2012-12044	N	LIT
KIIA 5.4.4 KIIA 5.10 (OECD)	Vlastos, D., Stivaktakis, P., Matthopoulos, D.P.	2006	Pesticide exposure and genotoxicity correlations within a Greek farmers' group International Journal of Environmental Analytical Chemistry 86, 215-223 GLP: N, published: Y 2310116 / ASB2012-12045	N	LIT
KIIA 5.4.4 KIIA 5.5.3 KIIA 5.10 KIIIA1 7.6.3 (OECD)	Williams, G.M., Kroes, R., Munro, I.C.	2000	Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans Regulatory Toxicology and Pharmacology 31, 117-165 GLP: N, published: Y 2310132 / ASB2012-12053	N	LIT
KIIA 5.4.4 (OECD)	[REDACTED]	1996	A micronucleus study in mice for the product GILFOS G1206096 ! G.1.2 - 60/96 TOX1999-253	N	---
KIIA 5.4.6 (OECD)	[REDACTED]	1992	Glyphosate technical (FSG 03090 H/05, March 1990): Dominant lethal test in wistar rats Report: 888-DLT ! TOXI-888/1992 ! ES-GPT-DLT, published: N, TOX9551102	N	---
KIIA 5.4.6 (OECD)	[REDACTED]	1980	Dominant lethal mutagenicity assay with technical Glyphosate in mice, Report: 401-064 ! IR-79-014, published: N, TOX9552377	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.5.1 KIIA 5.10 (OECD)	[REDACTED]	1996	Glyphosate Acid: One Year Dietary Toxicity Study in Rats CTL/P/5143 SYN GLP: Y, published: N 2309341 / TOX2000-1998	N	SYN
KIIA 5.5.2 (OECD)	[REDACTED]	1993	Glyphosate - 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) 7867 CHE GLP: Y, published: N 2309374 / TOX9750499	N	CHE
KIIA 5.5.2 KIIA 5.10 (OECD)	[REDACTED]	2001	Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats CTL/PR1111 SYN GLP: Y, published: N 2309368 / ASB2012-11488	N	SYN
KIIA 5.5.2 (OECD)	[REDACTED]	1974	2-year chronic oral toxicity study with CP 67573 in albino rats B564 ! BTL-71-32 Z35230	N	---
KIIA 5.5.2 (OECD)	[REDACTED]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 1 (Seite 1-500) IET 94-0150 Vol.1 ALS GLP: Y, published: N 2309360 / ASB2012-11484	N	ALS
KIIA 5.5.2 (OECD)	[REDACTED]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 2 (Seite 501-1000) IET 94-0150 Vol. 2 ALS GLP: Y, published: N 2309362 / ASB2012-11485	N	ALS
KIIA 5.5.2 (OECD)	[REDACTED]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol.3 (Seite 1001-1500) IET 94-0150 Vol. 3 ALS GLP: Y, published: N 2309364 / ASB2012-11486	N	ALS
KIIA 5.5.2 (OECD)	[REDACTED]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 4 (Seite 1501-2051) IET 94-0150 Vol. 4 ALS GLP: Y, published: N 2309366 / ASB2012-11487	N	ALS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.5.2 (OECD)	[REDACTED]	1990	Chronic study of glyphosate administered in feed to Albino rats MSL-10495 MON GLP: Y, published: N 2309384 / TOX9300244	N	MON
KIIA 5.5.2 (OECD)	[REDACTED]	1996	Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats TOXI:886.C.C-R FSG GLP: Y, published: N 2309343 / TOX9651587 / TOX9600015	N	FSG
KIIA 5.5.2 (OECD)	[REDACTED]	2009	Glyphosate Technical: Dietary combined chronic toxicity / carcinogenicity study in the rat SPL2060-0012 NUF GLP: Y, published: N 2309391 / ASB2012-11490	Y	NUF
KIIA 5.5.3 KIIA 5.10 (OECD)	Acquavella, J.F., Gustin, C., Alexander, B.H., Mandel, J.S.	2005	Implications for epidemiologic research on variation by pesticide in studies of farmers and their families Scandinavian Journal of Work Environment & Health 31, 105-109 GLP: N, published: Y 2309540 / ASB2012-11530	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Alavanja, M.C., Samanic, C., Dosemeci, M., Lubin, J., Tarone, R., Lynch, C.F., Knott, C., Thomas, K., Hoppin, J.A., Barker, J., Coble, J., Sandler, D.P., Blair, A.	2003	Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort Am J Epidemiol 157, 800-814 GLP: N, published: Y 2309554 / ASB2012-11535	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Andreotti, G., Freeman, L.E.B., Hou, L., Coble, J., Rusiecki, J., Hoppin, J.A., Silverman, D.T., Alavanja, M.C.R.	2009	Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort International Journal of Cancer 124, 2495-2500 GLP: N, published: Y 2309572 / ASB2012-11544	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.5.3 (OECD)	[REDACTED]	1993	Glyphosate: 104-week dietary carcinogenicity study in mice 7793 ! IRI 438618 TOX9552382	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Band, P.R., Abanto, Z., Bert, J., Lang, B., Fang, R., Gallagher, R.P., Le, N.D.	2011	Prostate Cancer Risk and Exposure to Pesticides in British Columbia Farmers Prostate 71, 168-183 GLP: N, published: Y 2309594 / ASB2012-11555	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Barale-Thomas, E.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 473–474 ASB2013-10998	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Berry, C.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 445–446 ASB2013-10988	N	--
KIIA 5.5.3 KIIA 5.10 (OECD)	Blair, A., Freeman, L.B.	2009	Epidemiologic Studies in Agricultural Populations: Observations and Future Directions Journal of Agromedicine 14, 125-131 GLP: N, published: Y 2309618 / ASB2012-11566	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Carreon, T., Butler, M.A., Ruder, A.M., Waters, M.A., Davis-King, K.E., Calvert, G.M., Schulte, P.A., Connally, B., Ward, E.M., Sanderson, W.T., Heinemann, E.D., Mandel, J.S., Morten, R.F., Reding, D.J., Rosenmann, K.D., Talaska, G.	2005	Gliomas and farm pesticide exposure in women: The Upper Midwest Health Study Environmental Health Perspectives 113, 546-551 GLP: N, published: Y 2309660 / ASB2012-11585	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.5.3 KIIA 5.10 (OECD)	McDuffie, H.H., Pahwa, P., McLaughlin, J.R., Spinelli, J.J., Fincham, S., Dosman, J.A., Robson, D., Skinnider, L.F., Choi, N.W.	2001	Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health Cancer Epidemiol Biomarkers Prev 10, 1155- 1163 GLP: N, published: Y 2309924 / ASB2011-364	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	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 161, 121- 135 GLP: N, published: Y 2309720 / ASB2012-11613	N	MOD
KIIA 5.5.3 KIIA 5.10 (OECD)	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 123, 1657-1663 GLP: N, published: Y 2309722 / ASB2012-11614	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Farmer, D.R., Lash, T.L., Acquavella, J.F.	2005	Glyphosate Results Revisited Environmental Health Perspectives 113, A365-A366 GLP: N, published: Y 2309726 / ASB2012-11616	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	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 112, 361- 635 GLP: N, published: Y 2309734 / ASB2012-11620	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Freeman, L.B.	2009	Evaluation of agricultural exposures: the agricultural health study and the agricultural cohort consortium Reviews on Environmental Health 24, 311- 318 GLP: N, published: Y 2309740 / ASB2012-11623	N	MOD

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.5.3 KIIA 5.10 (OECD)	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 162, 849- 857 GLP: N, published: Y 2309746 / ASB2012-11624	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	George, J., Prasad, S., Mahmood, Z., Shukla, Y.	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach J Proteomics 73, 951-964 GLP: N, published: Y 2309766 / ASB2012-11829	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Grunewald, W.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 447–448 ASB2013-11001	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Hammond, B.; Goldstein, D. A.; Saltmiras, D.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 459–464 ASB2013-10995	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Hardell, L., Eriksson, M.	1999	A case-control study of non-Hodgkin lymphoma and exposure to pesticides Cancer 85, 1353-1360 GLP: N, published: Y 2309788 / ASB2012-11838	N	MOD
KIIA 5.5.3 KIIA 5.10 (OECD)	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 Leukemia & Lymphoma 43, 1043-1049 GLP: N, published: Y 2309790 / ASB2012-11839	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Henemann, J. A.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 442 ASB2013-10987	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	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 17, 30-39 GLP: N, published: Y 2309844 / ASB2012-11865	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.5.3 (OECD)	[REDACTED]	1983	A chronic feeding study of glyphosate (Roundup technical) in mice 77-2061 ! (BDN-77-420) TOX9552381	N	---
KIIA 5.5.3 (OECD)	[REDACTED]	2001	Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice TOXI: 1559.CARCI-M FSG GLP: Y, published: N 2309396 / ASB2012-11491	Y	FSG
KIIA 5.5.3 KIIA 5.10 (OECD)	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 Blood 113, 6386-6391 GLP: N, published: Y 2309874 / ASB2012-11875	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Langridge, P.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 441 ASB2013-10986	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Lash, T.L.	2007	Bias analysis applied to Agricultural Health Study publications to estimate non-random sources of uncertainty J Occup Med Toxicol 2, 1-9 GLP: N, published: Y 2309876 / ASB2012-11877	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	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 61, 743-749 GLP: N, published: Y 2309888 / ASB2012-11883	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	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 62, 786-792 GLP: N, published: Y 2309886 / ASB2012-11882	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.5.3 KIIA 5.10 (OECD)	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 33, 293-303 GLP: N, published: Y 2309948 / ASB2012-11909	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Multigner, L., Ndong, J.R., Oliva, A., Blanchet, P.	2008	Environmental pollutants and prostate cancer: epidemiological data Gynecol Obstet Fertil 36, 848-856 GLP: N, published: Y 2309964 / ASB2012-11917	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Ndong, J.R., Blanchet, P., Multigner, L.	2009	Pesticides and prostate cancer epidemiological data Bulletin Du Cancer 96, 171-180 GLP: N, published: Y 2309974 / ASB2012-11922	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	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 TOX1999-687	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Ollivier, L.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 458 ASB2013-11000	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Pahwa, P., Karunanayake, C.P., Dosman, J.A., Spinelli, J.J., McDuffie, H.H., McLaughlin, J.R.	2010	Multiple Myeloma and Exposure to Pesticides: A Canadian Case-Control Study Journal of Agromedicine 17, 40-50 GLP: N, published: Y 2309996 / ASB2012-11987	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Panchin, A. Y.;	2013	Toxicity of roundup-tolerant genetically modified maize is not supported by statistical tests Food and Chemical Toxicology 53 (2013) 475 ASB2013-10937	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Pilu, R.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 454 ASB2013-10992	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.5.3 KIIA 5.10 (OECD)	De Roos, A.J., Blair, A., Rusiecki, J.A., Hoppin, J.A., Svec, M., Dosemeci, M., Sandler, D.P., Alavanja, M.C.	2005	Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study Environmental Health Perspectives 113, 49-54 GLP: N, published: Y 2309704 / ASB2012-11605	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	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 60 GLP: N, published: Y 2309706 / ASB2012-11606	N	LIT
KIIA 5.5.3 KIIA 5.10 (OECD)	Schorsch, F.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 465–466 ASB2013-10996	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Séralini, G.-E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Spiroux de Vendômois, J.;	2012	Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food and Chem Toxicol., in Press, ASB2012-15514	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Séralini, G. E.; Mesnage, R.; Defarge, N.; Gress, S.; Hennequin, D.; Clair, E.; Malatesta, M.; Spiroux de Vendômois, J.;	2013	Answers to critics: Why there is a long term toxicity due to a Rounduptolerant genetically modified maize and to a Roundup herbicide Food and Chemical Toxicology 53 (2013) 476–483 ASB2013-10985	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	de Souza, L.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 440 ASB2013-10999	N	---
KIIA 5.5.3 (OECD)		1997	HR-001: 18-Month Oral Oncogenicity Study in Mice IET 940151 ALS GLP: Y, published: N 2309415 / ASB2012-11493	Y	ALS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.5.3 KIIA 5.10 (OECD)	Tester, M.;	2012	Letter to the Editor Food and Chemical Toxicology 53 (2013) 457 ASB2013-10994	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Tien, D. L.; Huy, H. L.;	2012	Comments on “Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize” Food and Chemical Toxicology 53 (2013) 443–444 ASB2013-10984	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Trewavas, A.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 449 ASB2013-10989	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Tribe, D.;	2012	Letter to the editor Food and Chemical Toxicology 53 (2013) 467–472 ASB2013-10997	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Wager, R.;	2013	Letter to the editor Food and Chemical Toxicology 53 (2013) 455–456 ASB2013-10993	N	---
KIIA 5.5.3 KIIA 5.10 (OECD)	Weichenthal, S., Moase, C., Chan, P.	2010	A review of pesticide exposure and cancer incidence in the Agricultural Health Study cohort Environ Health Perspect 118, 1117-1125 GLP: N, published: Y 2310122 / ASB2012-12048	N	LIT
KIIA 5.5.3 (OECD)		2009	Glyphosate Technical: Dietary carcinogenicity study in the mouse SPL 2060-0011 NUF GLP: Y, published: N 2309412 / ASB2012-11492	Y	NUF
KIIA 5.6.1 (OECD)		1985	Three-generation reproduction study in rats with the oral administration of glyphosate TOX9650161	N	---
KIIA 5.6.1 (OECD)		1988	Report on effect of glyphosate technical of Excel Industries Ltd., Bombay, on fertility and general reproductive performance (Segment I) TOX9551832	N	---
KIIA 5.6.1 (OECD)		1988	Report on effect of pesticides on reproductive process - Segment IV - three generation reproduction study with albino rats using glyphosate technical of Excel Industries Ltd., Bombay TOX9551965	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.6.1 (OECD)	[REDACTED]	1991	Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring CHV 42/90619 TOX9552388	N	---
KIIA 5.6.1 (OECD)	[REDACTED]	1992	The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat CHV 47/911129 CHE GLP: Y, published: N 2309436 / TOX9552389	N	CHE
KIIA 5.6.1 (OECD)	D [REDACTED]	2007	Glyphosate technical: Dietary Two-Generation Reproduction Study in the Rat 2060/0013 NUF GLP: Y, published: N 2309418 / ASB2012-11494	Y	NUF
KIIA 5.6.1 (OECD)	[REDACTED]	2000	Glyphosate acid: Multigeneration reproduction toxicity study in rats CTL/P/6332 SYN/ MON GLP: Y, published: N 2309423 / TOX2000-2000	Y	SYN
KIIA 5.6.1 (OECD)	[REDACTED]	1990	Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats MSL-10387 MON GLP: Y, published: N 2309439 / ASB2012-11496 / TOX9552387	N	MON
KIIA 5.6.1 (OECD)	[REDACTED]	1993	Two Generation Reproduction Study in Wistar Rats TOXI: 885-RP-G2 GLP: Y, published: N 2309427 / TOX9300009 / TOX9551104	N	FSG
KIIA 5.6.1 (OECD)	[REDACTED]	1997	HR-001: A two-generation reproduction study in rats IET 96-0031 ALS GLP: Y, published: N 2309425 / ASB2012-11495	N	ALS
KIIA 5.6.10 (OECD)	Antoniou, M.; Habib, M.E.M.; Howard, C.V.; Jennings, R.C.; Leifert, C.; Nodari, R.O.; Robinson, C.J.; Fagan, J.	2012	Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence J Environ Anal Toxicol 2012, S:4, ASB2012-15927	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.6.10 (OECD)	[REDACTED]	1991	The effect of glyphosate on pregnancy of the rat (incorporates preliminary investigation) CHV 43 u. 41/90716 TOX9552393	N	---
KIIA 5.6.10 (OECD)	[REDACTED]	1995	HR-001: Teratogenicity Study in Rats IET 94-0152 ALS GLP: Y, published: N 2309444 / ASB2012-11497	Y	ALS
KIIA 5.6.10 (OECD)	[REDACTED]	2012	Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity Toxicology, in Press ASB2012-13917	N	---
KIIA 5.6.10 (OECD)	[REDACTED]	2002	Amendment 001 to glyphosate acid: Developmental toxicity study in the rat CTL/P/4819 ! RR0690 ASB2012-10080	N	---
KIIA 5.6.10 (OECD)	[REDACTED]	1991	Glyphosate techn. (ESG 03090 H/05 March 1990): Teratogenicity study in Wistar rats ES.883.TER-R ! TOXI-883/1991 ! ES-GPT-TER-R TOX9551195	N	---
KIIA 5.6.10 (OECD)	[REDACTED]	1980	Glyphosate: Teratology study in rats 401-054 ! IR-79-016 TOX9552392	N	---
KIIA 5.6.11 (OECD)	[REDACTED]	2013	No evidence of endocrine disruption by glyphosate in male and female pubertal assays. Abstract ASB2013-3464	N	---
KIIA 5.6.11 (OECD)	[REDACTED]	1989	Rabbit Teratology Study with Glyphosate Technical IIT Project No. 1086 EXC GLP: Y, published: N 2309462 / TOX9551960	N	EXC
KIIA 5.6.11 (OECD)	[REDACTED]	1991	The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations) CHV 45 & 39 & 40/901 CHE GLP: Y, published: N 2309454 / TOX9552391	N	CHE
KIIA 5.6.11 (OECD)	[REDACTED]	1996	Glyphosate technical: Oral gavage teratology study in the rabbit 434/020 NUF GLP: Y, published: N 2309448 / ASB2012-11499	Y	NUF

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.6.11 (OECD)		1995	HR-001: A Teratogenicity Study in Rabbits IET 94-0153 ALS GLP: Y, published: N 2309446 / ASB2012-11498	N	ALS
KIIA 5.6.11 (OECD)	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 Critical Reviews in Toxicology 43(2013)279- 95, ASB2013-3462	N	---
KIIA 5.6.11 (OECD)		1996	Glyphosate acid: Developmental toxicity study in the rabbit CTL/P/5009 SYN GLP: Y, published: N 2309450 / TOX2000-2002	N	SYN
KIIA 5.6.11 (OECD)		1993	Teratogenicity study in rabbits - Tets compound: Glyphosate technical TOXI: 884-TER-RB GLP: Y, published: N 2309457 / TOX9551106	N	FSG
KIIA 5.6.11 (OECD)		1980	Technical Glyphosate: Teratology study in rabbits IR-79-018 MON GLP: N, published: N 2309452 / TOX9552390	N	MON
KIIA 5.6.2 (OECD)		1996	Glyphosate acid: Developmental toxicity study in the rat 29.03.1996 CTL/P/4819 ! RR 0690 TOX2000-2001	N	---
KIIA 5.7. (OECD)		1996	Glyphosat Acid: Acute delayed neurotoxicity study with in the domestic hen CTL/C/3122 ! C2.8/01 ! ISN 361 ASB2013-9828	N	---
KIIA 5.7.1 (OECD)		1996	Glyphosate acid: Acute neurotoxicity study in rats CTL/P/4866 SYN GLP: Y, published: N 2309464 / ASB2012-11500	N	SYN
KIIA 5.7.4 KIIA 5.10 (OECD)	Astiz, M., de Alaniz, M.J., Marra, C.A.	2009	Effect of pesticides on cell survival in liver and brain rat tissues Ecotoxicol Environ Saf 72, 2025-2032 GLP: N, published: Y 2309582 / ASB2012-11549	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.7.4 KIIA 5.9 KIIA 5.10 (OECD)	Barbosa, E.R., da Costa, M.D.L., Bacheschi, L.A., Scaff, M., Leite, C.C.	2001	Parkinsonism after glycine-derivate exposure Movement Disorders 16, 565-568 GLP: N, published: Y 2309598 / ASB2012-11557	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	Cole, R.D., Anderson, G.L., Williams, P.L.	2004	The nematode Caenorhabditis elegans as a model of organophosphate-induced mammalian neurotoxicity Toxicology and Applied Pharmacology 194, 248-256 GLP: N, published: Y 2309680 / ASB2012-11594	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	da Costa, M.D.L., Goncalves, L.R., Barbosa, E.R., Bacheschi, L.A.	2003	Neuroimaging abnormalities in parkinsonism: study of five cases Arquivos De Neuro-Psiquiatria 61, 381-386 GLP: N, published: Y 2309688 / ASB2012-11598	N	LIT
KIIIA 5.7.4 KIIA 5.10 (OECD)	Engel, L.S., Checkoway, H., Keifer, M.C., Seixas, N.S., Longstreth, W.T., Jr., Scott, K.C., Hudnell, K., Anger, W.K., Camicioli, R.	2001	Parkinsonism and occupational exposure to pesticides Occup Environ Med 28, 582-589 GLP: N, published: Y 2309718 / ASB2012-11612	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	Gui, Y.-x., Fan, X.-n., Wang, H.-m., Wang, G., Chen, S.-d.	2012	Glyphosate induced cell death through apoptotic and autophagic mechanisms Neurotoxicology and Teratology GLP: N, published: Y 2309778 / ASB2012-11835	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	Heu, C., Elie- Caille, C., Mougey, V., Caunay, S., Nicod, L.	2012	A step further toward glyphosate-induced epidermal cell death: Involvement of mitochondrial and oxidative mechanisms Environmental Toxicology and Pharmacology 34, 144-153 GLP: N, published: Y 2309800 / ASB2012-11844	N	LIT
KIIA 5.7.4 (OECD)		1996	Glyphosate Acid: Subchronic Neurotoxicity Study In Rats CTL/P/4867 SYN GLP: Y, published: N 2309466 / ASB2012-11501	N	SYN

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.7.4 KIIA 5.10 (OECD)	Kamel, F., Tanner, C.M., Umbach, D.M., Hoppin, J.A., Alavanja, M.C.R., Blair, A., Comyns, K., Goldman, S.M., Korell, M., Langston, J.W., Ross G.W., Sandler, D.P.	2007	Pesticide exposure and self-reported Parkinson's disease in the agricultural health study American Journal of Epidemiology 165, 364- 374 GLP: N, published: Y 2309838 / ASB2012-11862	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	Mink, P.J., Mandel, J.S., Lundin, J.I., Scurman, B.K.	2011	Epidemiologic studies of glyphosate and non- cancer health outcomes: A review Regulatory Toxicology and Pharmacology 61, 172-184 GLP: N, published: Y 2309938 / ASB2012-11904	N	LIT
KIIA 5.7.4 KIIA 5.10 (OECD)	Negga, R., Rudd, D.A., Davis, N.S., Justice, A.N., Hatfield, H.E., Valente, A.L., Fields, A.S., Fitsanakis, V.A.	2011	Exposure to Mn/Zn ethylene-bis- dithiocarbamate and glyphosate pesticides leads to neurodegeneration in Caenorhabditis elegans NeuroToxicology 32, 331-341 GLP: N, published: Y 2309976 / ASB2012-11923	N	LIT
KIIA 5.7.4 KIIA 5.9 KIIA 5.10 (OECD)	Wang, G., Fan, X.N., Tan, Y.Y., Cheng, Q., Chen, S.D.	2011	Parkinsonism after chronic occupational exposure to glyphosate Parkinsonism & Related Disorders 17, 486- 487 GLP: N, published: Y 2310120 / ASB2012-12047	N	LIT
KIIA 5.8 (OECD)		1996	AMPA, Reverse Mutation Test IET 96-0076 ALS GLP: Y, published: N 2309478 / ASB2012-11507	Y	ALS
KIIA 5.9 (OECD)	Bakke, J. P.	1991	Evaluation of the potential of AMPA to induce unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay using the male F-344 rats 2495-V01-91 ! SR-91-234 TOX9552409	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.8 (OECD)	Callander, R.D.	1988	Aminomethyl Phosphonic Acid: An Evaluation of Mutagenic Potential Using <i>S. typhimurium</i> and <i>E. coli</i> CTL/P/2206 SYN GLP: Y, published: N 2309476 / TOX9500043	N	SYN
KIIA 5.8 (OECD)	[REDACTED]	1993	AMPA: Acute oral toxicity (limit) test in rats 8763 ! IRI 552409 TOX9552395	N	---
KIIA 5.8 (OECD)	[REDACTED]	1993	AMPA: Acute dermal toxicity (limit) test in rats 8764 ! IRI 552409 TOX9552396	N	---
KIIA 5.8 (OECD)	[REDACTED]	1993	AMPA: Magnusson-Kligman maximisation test in guinea pigs 8765 ! IRI 552409 TOX9300374	N	---
KIIA 5.8 (OECD)	[REDACTED]	1979	CP 50435: 90-day subacute rat toxicity study 401-050 ! IRD-78-174 TOX9552401	N	---
KIIA 5.8 (OECD)	[REDACTED]	1992	AMPA: Teratogenicity study in rats 7891 ! IRI 490421 TOX9300348	N	---
KIIA 5.8 (OECD)	[REDACTED]	1993	AMPA: 4 week dose range finding study in rats with administration by gavage 8803 ! IRI 450860 TOX9300349	N	---
KIIA 5.8 (OECD)	[REDACTED]	1991	A developmental toxicity study of AMPA in rats WIL-50159 ! WI-90-266 TOX9552414	N	---
KIIA 5.8 (OECD)	[REDACTED]	1991	Assessment of acute oral toxicity of (N-methyl-N-phosphonomethyl)glycine to rats 12837 TOX9552398	N	---
KIIA 5.8 (OECD)	[REDACTED]	1993	Mutagenicity test: Ames salmonella test with AMPA, batch 286-JRJ-73-4, 13269 TOX9300378	N	---
KIIA 5.8 (OECD)	[REDACTED]	1993	AMPA, batch 286-JRJ-73-4: Mutagenicity test: <i>In vitro</i> mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y) 13270 TOX9300380	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.8 (OECD)	[REDACTED]	1993	Mutagenicity test: Micronucleus test with AMPA, batch 286-JRJ-73-4 13268 TOX9300379	N	---
KIIA 5.8 (OECD)	[REDACTED]	1993	Mouse micronucleus study of AMPA EHL-90170/ML-90-404 ! MSL 13243 TOX9552413	N	---
KIIA 5.8 (OECD)	[REDACTED]	1996	AMPA: Acute Oral Toxicity Study In Mice IET 96-0075 ALS GLP: Y, published: N 2309468 / ASB2012-11502	Y	ALS
KIIA 5.8 (OECD)	[REDACTED]	1988	Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat CTL/P/2266 SYN GLP: Y, published: N 2309470 / TOX9500044	N	SYN
KIIA 5.8 (OECD)	[REDACTED]	2002	Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration - LIMIT TEST 16168/02 GLP: Y, published: N 2309472 / ASB2012-11503	Y	FSG
KIIA 5.8 (OECD)	[REDACTED]	2002	Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitisation Test in Guinea Pigs according to Magnusson And Kligman (Maximisation Test) 16169/02 GLP: Y, published: N 2309474 / ASB2012-11506	Y	FSG
KIIA 5.8 (OECD)	[REDACTED]	2002	Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes in vitro procedure with AMPA (Amino methyl phosphonic acid) IPL-R 020625 ALS GLP: Y, published: N 2309480 / ASB2012-11508	Y	ALS
KIIA 5.8 (OECD)	[REDACTED]	1993	AMPA: 13-week toxicity study in rats with administration by gavage 7866 ! IRI 450876 TOX9300377	N	---
KIIA 5.8 (OECD)	[REDACTED]	1991	90-day oral (capsule) toxicity study in dogs with AMPA WIL-50173 ! WI-90-354 TOX9552406	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.9 (OECD)	Acquavella, J.F., Weber, J.A., Cullen, M.R., Cruz, O.A., Martens, M.A., Holden, L.R., Riordan, S., Thompsen, M., Farmer, D.	1999	Human ocular effects from self-reported exposures to Round-up herbicides Human & Experimental Toxicology (paper) 18, 479-486 GLP: N, published: Y 2309482 / TOX2002-699	N	LIT
KIIA 5.9 (OECD)	Bando, H., Murao, Y., Aoyagi, U., Hirakawa, A., Iwase, M., Nakatani, T.	2010	[Extreme hyperkalemia in a patient with a new glyphosate potassium herbicide poisoning: report of a case] Chudoku Kenkyu 23, 246-249 GLP: N, published: Y 2309596 / ASB2012-11556	N	LIT
KIIA 5.9 KIIIA1 7.6.3 (OECD)	Bradberry, S.M., Proutfoot, A.T., Vale, J.A.	2004	Glyphosate poisoning Toxicological reviews (paper), 23, 159-167 GLP: N, published: Y 2309484 / ASB2012-11509	N	LIT
KIIA 5.9 (OECD)	Bradberry, S.M., Proudfoot, A.T., Vale, J.A.	2004	Glyphosate poisoning Toxicol Rev 23, 159-167 GLP: N, published: Y 2309642 / ASB2012-11576	N	LIT
KIIA 5.9 (OECD)	Burger, R.; Begemann, K.; Meyer, H.; Hahn, A.;	2009	Severe dyspnoea after spraying of a pesticide containing glyphosate. Lung damage histologically confirmed Clinical Toxicology (2009) 47, 506 ASB2013-11831	N	---
KIIA 5.9 (OECD)	Chang, C.-J., Peng, Y.-C., Hung, W.-H., Yang, D.-Y., Lin, T.-J.	1999	Clinical impact of upper gastrointestinal tract injuries in glyphosate-surfactant oral intoxication Human & Experimental Toxicology (paper), 18, 475-478 GLP: N, published: Y 2309486 / ASB2012-11510	N	LIT
KIIA 5.9 (OECD)	Goldstein, D.A., Johnson, G., Farmer, D.R., Martens, M.A.	1999	Pneumonitis and herbicide exposure Chest (paper), 16, 1139-1140 GLP: N, published: Y 2309490 / ASB2012-11511	N	LIT
KIIA 5.09 (OECD)	Goldstein, D.A., Acquavella, J.F., Mannion, R.M., Farmer, D.R.	2002	An analysis of glyphosate data from the California Environmental Protection Agency Pesticide Illness Surveillance Program Journal of Toxicology-Clinical Toxicology 40, 885-892 GLP: N, published: Y 2309770 / ASB2012-11831	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.9 (OECD)	Kamijo, Y., Mekari, M., Yoshimura, K., Kano, T., Soma, K.	2012	Glyphosate-surfactant herbicide products containing glyphosate potassium salt can cause fatal hyperkalemia if ingested in massive amounts Clinical Toxicology 50, 159 GLP: N, published: Y 2309840 / ASB2012-11863	N	LIT
KIIA 5.9 (OECD)	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) 7, 906-910 GLP: N, published: Y 2309492 / ASB2012-11512	N	LIT
KIIA 5.9 (OECD)	Lee, C.H., Shih, C.P., Hsu, K.H., Hung, D.Z., Lin, C.C.	2008	The early prognostic factors of glyphosate-surfactant intoxication Am J Emerg Med 26, 275-281 GLP: N, published: Y 2309880 / ASB2012-11879	N	LIT
KIIA 5.9 KIIA 5.10 KIIIA1 7.6.3 (OECD)		1987	Irritating effect of glyphosate, surfactant and roundup on stomach and small intestine in dogs MON GLP: N, published: N 2309496 / TOX9552430	N	MON
KIIA 5.9 (OECD)	Paumgarten, F.J.R.	2012	ANVISA - Glyphosate Intoxications 2010 to 2012 in Brasil; ASB2013-13413	N	---
KIIA 5.9 (OECD)	Pushnoy, L.A., Avnon, L.S., Carel, R.S.	1998	Herbicide (Roundup) Pneumonitis Chest (paper), 114, 1769-1771 GLP: N, published: Y 2309498 / ASB2012-11513	N	LIT
KIIA 5.9 (OECD)	Sawada, Y., Nagai, Y., Ueyama, M., Yamamoto, I.	1988	Probable toxicity of surface-active agent in commercial herbicide containing glyphosate The Lancet (paper) 1, 299-301 GLP: N, published: Y 2309504 / Z35532	N	LIT
KIIA 5.9 (OECD)	Fominack, R., Conner, P., Yamashita, M.	1989	Clinical Management of Roundup® herbicide exposure The Japanese Journal of Toxicology (paper) 2, 187-192 GLP: N, published: Y 2309506 / TOX9552426	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	AFSSA	2009	Avis de l'Agence française de sécurité sanitaire des aliments relatif au glyphosate et aux préparations phytopharmaceutiques à base de cette substance active GLP: N, published: Y 2309546 / ASB2012-11532	N	LIT
KIIA 5.10 (OECD)		1996	Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat CTL/P/5160 SYN GLP: Y, published: N 2309518 / ASB2012-11520	N	SYN
KIIA 5.10 (OECD)		1996	Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat CTL/P/5160 SYN GLP: Y, published: N 2309558 / ASB2012-11557	Y	MOD
KIIA 5.10 (OECD)	Anonymous	2009	Final List of Initial Pesticide Active Ingredients and Pesticide Inert Ingredients to be Screened Under the Federal Food, Drug, and Cosmetic Act Federal Register /Vol. 74, No. 71 /Wednesday, April 15, 2009 /Notices, 17579-17585 74, 17579-17585 GLP: N, published: Y 2310108 / ASB2012-12041	N	LIT
KIIA 5.10 (OECD)	Anonymous	2013	Effects on ruminants and other herbivores (livestock and wild life) ASB2013-11007	N	---
KIIA 5.10 (OECD)	Arbuckle, T.E., Lin, Z.Q., Mery, L.S.	2001	An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population Environmental Health Perspectives 109, 851-857 GLP: N, published: Y 2309574 / ASB2012-11545	N	LIT
KIIA 5.10 (OECD)	Aris, A., Leblanc, S.	2011	Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada Reproductive Toxicology 31, 528-533 GLP: N, published: Y 2309578 / ASB2012-11547	N	LIT
KIIA 5.10 (OECD)	Axelrad, J.C., Howard, C.V., McLean, W.G.	2003	The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon Toxicology 185, 67-78 GLP: N, published: Y 2309590 / ASB2012-11553	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	Bailey, J.; Hauswirth, J.; Stump, D.;	2013	No evidence of endocrine disruption by glyphosate in male and female pubertal assays. Abstract SOT 2013 Annual Meeting, PS 1937: p 412 ASB2013-3464	N	---
KIIA 5.10 (OECD)	Bell, E.M., Hertz-Picciotto, I., Beaumont, J.J.	2001	A case-control study of pesticides and fetal death due to congenital anomalies Epidemiology 12, 148-156 GLP: N, published: Y 2309602 / ASB2012-11559	N	LIT
KIIA 5.10 (OECD)	Belle, R., Le Bouffant, R., Morales, J., Cosson, B., Cormier, P., Mulner- Lorillon, O.	2007	Sea urchin embryo, DNA-damaged cell cycle checkpoint and the mechanisms initiating cancer development J Soc Biol 201, 317-327 GLP: N, published: Y 2309604 / ASB2012-11560	N	LIT
KIIA 5.10 (OECD)	Benachour, N., Sipahutar, H., Moslerni, S., Gasnier, C., Travert, C., Seralini, G.E.	2007	Time- and dose-dependent effects of roundup on human embryonic and placental cells Archives of Environmental Contamination and Toxicology 53, 126-133 GLP: N, published: Y 2309608 / ASB2009-9018	N	LIT
KIIA 5.10 (OECD)	Benedetti, A.L., Vituri, C.D., Trentin, A.G., Domingues, M.A.C., Alvarez-Silva, M.	2004	The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb (R) Toxicology Letters 153, 227-232 GLP: N, published: Y 2309610 / ASB2012-11562	N	LIT
KIIA 5.10 (OECD)	Benitez-Leite, S., Macchi, M., Acosta, M.	2009	Malformaciones congénitas asociadas a agrotóxicos Archives of Pediatrics 80 (3):377-378. 80, 377-378 GLP: N, published: Y 2309612 / ASB2012-11563	N	LIT
KIIA 5.10 (OECD)	Beuret, C.J., Zirulnik, F., Gimenez, M.S.	2005	Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses Reproductive Toxicology 19, 501-504 GLP: N, published: Y 2309614 / ASB2012-11564	N	LIT
KIIA 5.10 (OECD)	BfR	2009	BfR-Bewertung der Studie "Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines" vom 06.08.2009 GLP: N, published: Y 2309616 / ASB2012-11565	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	[REDACTED]	1987	Synergism and potentiation in rats of Glyphosate (tech.) of Excel Industries Ltd., Bombay TOX9551964	N	---
KIIA 5.10 (OECD)	Blair, A., Zahm, S.H.	1993	Patterns of pesticide use among farmers: implications for epidemiologic research Epidemiology 4, 55-62 GLP: N, published: Y 2309620 / ASB2012-11567	N	LIT
KIIA 5.10 (OECD)	Bleeke, M.S., Kurtzweil, M.L., Saltmiras, D.A.	2010	Dietary Exposure Assessment of Polyoxyethylenealkylamines (POEA) Surfactants MON GLP: N, published: Y 2309622 / ASB2010-6123	N	LIT
KIIA 5.10 (OECD)	BVL	2010	Glyphosate - Comments from Germany on the paper by Paganelli, A. et al. (2010): "Glyphosate-based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling" GLP: N, published: Y 2309648 / ASB2012-11579	N	LIT
KIIA 5.10 (OECD)	Campaña, H.; Pawluk, M. S.; López Camelo, J. S.; Grupo de Estudio del ECLAMC	2010	Prevalencia al nacimiento de 27 anomalías congénitas seleccionadas, en 7 regiones geográficas de la Argentina. Births prevalence of 27 selected congenital anomalies in 7 geographic regions of Argentina Arch Argent Pediatr 2010;108(5):409-417 ASB2013-10559	N	---
KIIA 5.10 (OECD)	Clair, E., Linn, L., Traver, C., Amiel, C., Seralini, G.-E., Panoff, J.-M.	2012	Effects of Roundup and Glyphosate on Three Food Microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus Current Microbiology 64, 486-491 GLP: N, published: Y 2309674 / ASB2012-11592	N	LIT
KIIA 5.10 (OECD)	Clair, E., Mesnage, R., Traver, C., Seralini, G.-E.	2012	A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells <i>in vitro</i> , and testosterone decrease at lower levels Toxicology in Vitro 26, 269-279 GLP: N, published: Y 2309678 / ASB2012-1628	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 KIIIA1 7.6.3 (OECD)	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 Toxicology Letters 142, 45-52 GLP: N, published: Y 2309692 / ASB2012-11600	N	LIT
KIIA 5.10 KIIIA1 7.6.3 (OECD)	Dallegrave, E., Mantese, F.D., Oliveira, R.T., Andrade, A.J.M., Dalsenter, P.R., Langeloh, A.	2007	Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats Archives of Toxicology 81, 665-673 GLP: N, published: Y 2309694 / ASB2012-2721	N	LIT
KIIA 5.10 (OECD)	Daruich, J., Zirulnik, F., Gimenez, M.S.	2001	Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses Environmental Research 85, 226-231 GLP: N, published: Y 2309696 / ASB2012-11601	N	LIT
KIIA 5.10 (OECD)	European Commission	2011	Standing Committee on the Food Chain and Animal Health, Section Phytopharmaceuticals - Plant Protection Products - Legislation - 22-23 November 2010 GLP: N, published: Y 2309724 / ASB2012-11615	N	LIT
KIIA 5.10 (OECD)	Forgacs, A.L., Ding, Q., Jaremba, R.G., Huhtaniemi, I.T., Rahman, N.A., Zacharewski, T.R.	2012	BLTK1 Murine Leydig Cells: A Novel Steroidogenic Model for Evaluating the Effects of Reproductive and Developmental Toxicants Toxicological Sciences GLP: N, published: Y 2309736 / ASB2012-11621	N	LIT
KIIA 5.10 (OECD)	French Committee for the Study of Toxicity	2005	Enquiry into the referral of the Committee for the Study of Toxicity by the DGAL regarding the article "Differential effects of glyphosate and Roundup on human placental cells and aromatase." Richard S., Moslemi S., Sipahutar H., Benachour GLP: N, published: Y 2309742 / ASB2009-9025	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	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 110:441-449 110, 441-449 GLP: N, published: Y 2309750 / ASB2012-11626	N	LIT
KIIA 5.10 (OECD)	Garry, V.F., Holland, S.E., Erickson, L.L., Burroughs, B.L.	2003	Male reproductive hormones and thyroid function in pesticide applicators in the Red River Valley of Minnesota Journal of Toxicology and Environmental Health-Part A 66, 965-986 GLP: N, published: Y 2309752 / ASB2012-11627	N	LIT
KIIA 5.10 (OECD)	Gasnier, C., Benachour, N., Clair, E., Travert, C., Langlois, F., Laurant, C., Decroix- Laporte, C., Seralini, G.E.	2010	Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines J Occup Med Toxicol 5 GLP: N, published: Y 2309754 / ASB2012-11628	N	LIT
KIIA 5.10 (OECD)	Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.C., Seralini, G.E.	2010	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines Toxicology 262, 184-191 GLP: N, published: Y 2309756 / ASB2012-11629	N	LIT
KIIA 5.10 (OECD)	Gasnier, C., Laurant, C., Decroix- Laporte, C., Mesnage, R., Clair, E., Travert, C., Seralini, G.E.	2011	Defined plant extracts can protect human cells against combined xenobiotic effects J Occup Med Toxicol 6, 3 GLP: N, published: Y 2309758 / ASB2012-11630	N	LIT
KIIA 5.10 (OECD)	[REDACTED]	2010	An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats WIL-50361 GTF GLP: Y, published: N 2309782 / ASB2012-11519	Y	MOD

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)		2012	Glyphosate - A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice WIL-50393 MON GLP: Y, published: N 2309522 / ASB2012-11521	Y	EGT
KIIA 5.10 (OECD)	Hecker, M., Hollert, H., Cooper, R., Vinggaard, A.M., Akahori, Y., Murphy, M., Nellemann, C., Higley, E., Newsted, J., Laskey, J., Buckalew, A.	2011	The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter- laboratory validation study Environmental Science and Pollution Research 18, 503-515 GLP: N, published: Y 2309792 / ASB2012-11840	N	LIT
KIIA 5.10 (OECD)	Heu, C., Berquand, A., Elie-Caille, C., Nicod, L.	2012	Glyphosate-induced stiffening of HaCaT keratinocytes, a Peak Force Tapping study on living cells Journal of structural biology 178, 1-7 GLP: N, published: Y 2309798 / ASB2012-11843	N	LIT
KIIA 5.10 (OECD)	Hokanson, R., Fudge, R., Chowdhary, R., Busbee, D.	2007	Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate Hum Exp Toxicol 26, 747-752 GLP: N, published: Y 2309804 / ASB2012-11846	N	LIT
KIIA 5.10 (OECD)	Kelce, W.R., Lamb, J.C., DeSesso, J.M.	2010	A Critique of prepubertal exposure to commercial formulation of the herbicide glyphosate GLP: N, published: Y 2309848 / ASB2012-11867	N	LIT
KIIA 5.10 (OECD)	Krüger, M.; Schrödl, W.; Neuhaus, J.; Shehata, A. A.;	2013	Field investigations of glyphosate in urine of Danish dairy cows J Environ Anal Toxicol, 3:5 ASB2013-11599	N	---
KIIA 5.10 (OECD)	Krüger, M.; Shehata, A. A.; Schrödl, W.; Rodloff, A.;	2013	Glyphosate suppresses the antagonistic effect of <i>Enterococcus</i> spp. on <i>Clostridium</i> <i>botulinum</i> in Press, Anaerobe xxx (2013) 1e5 ASB2013-8527	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	Krüger, M.; Große-Herrenthey, A.; Schrödl, W.; Gerlach, A.; Rodloff, A.;	2012	Visceral botulism at dairy farms in Schleswig Holstein, Germany - Prevalence of <i>Clostridium botulinum</i> in feces of cows, in animal feeds, in feces of the farmers, and in house dust Anaerobe 18 (2012) 221-223 ASB2013-13312	N	---
KIIA 5.10 (OECD)	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 Advances in Molecular Toxicology, Volume 6, 41-75 doi.org/10.1016/B978-0-444-59389-4.00002-1 ASB2013-10534	N	---
KIIA 5.10 (OECD)	Manas, F., Peralta, L., Raviolo, J., Ovando, H.G., Weyers, A., Ugnia, L., Cid, M.G., Larripa, I., Gorla, N.	2009	Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests Ecotoxicology and Environmental Safety 72, 834-837 GLP: N, published: Y 2309906 / ASB2012-11891	N	LIT
KIIA 5.10 (OECD)	Mandel, J.S., Alexander, B.H., Baker, B.A., Acquavella, J.F., Chapman, P., Honeycutt, R.	2005	Biomonitoring for farm families in the farm family exposure study Scand J Work Environ Health 31, 98-104 GLP: N, published: Y 2309910 / ASB2012-11893	N	LIT
KIIA 5.10 (OECD)	Marc, J.; Mulner-Lorillon, O.; Boulben, S. et al.	2002	Pesticide Roundup provokes cell division dysfunction at the level of CDK1/Cyclin B activation Chemical Research in Toxicology 15:326-331 (2002) ASB2013-9838	N	---
KIIA 5.10 (OECD)	Marc, J., Belle, R., Morales, J., Cormier, P., Mulner-Lorillon, O.	2004	Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition Toxicological Sciences 82, 436-442 GLP: N, published: Y 2309912 / ASB2012-11894	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	Marc, J., Mulner-Lorillon, O., Belle, R.	2004	Glyphosate-based pesticides affect cell cycle regulation Biology of the Cell 96, 245-249 GLP: N, published: Y 2309914 / ASB2009-9014	N	LIT
KIIA 5.10 (OECD)	Marc, J., Mulner-Lorillon, O., Durand, G., Belle, R.	2003	Embryonic cell cycle for risk assessment of pesticides at the molecular level Environmental Chemistry Letters 1, 8-12 GLP: N, published: Y 2309916 / ASB2009-9013	N	LIT
KIIA 5.10 (OECD)	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 GLP: N, published: Y 2309926 / ASB2012-11898	N	LIT
KIIA 5.10 (OECD)	Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A., Séralini, G.E.	2012	Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide Journal of Applied Toxicology:n/a-n/a GLP: N, published: Y 2309930 / ASB2012-11900	N	LIT
KIIA 5.10 (OECD)	Mose, T., Kjaerstad, M.B., Mathiesen, L., Nielsen, J.B., Edelfors, Sn., Knudsen L.E.	2008	Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system Journal of Toxicology and Environmental Health-Part a-Current Issues 71, 984-991 GLP: N, published: Y 2309958 / ASB2012-11914	N	LIT
KIIA 5.10 (OECD)	Mulet, J.M.	2011	Letter to the Editor Regarding the Article by Paganelli et al. Chemical Research in Toxicology 24, 609 GLP: N, published: Y 2309962 / ASB2012-11916	N	MOD
KIIA 5.10 (OECD)	Paumgarten, F.J.R., Cromonese, C., Freiere, C., Meyer, A., Koiman, S.	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, out, 2012 ASB2013-10538	N	---
KIIA 5.10 (OECD)	Paganelli, A., Gnazzo, V., Acosta, H., Lopez, S.L., Carrasco, A.E.	2010	Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling Chem Res Toxicol 23, 1586-1595 GLP: N, published: Y 2309994 / ASB2012-11986, ASB2010-11410	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	Palma, G.	2011	Letter to the Editor Regarding the Article by Paganelli et al. Chemical Research in Toxicology 24, 775-776 GLP: N, published: Y 2310000 / ASB2012-11989	N	LIT
KIIA 5.10 (OECD)	Quassinti, L., Maccari, E., Murri, O., Bramucci, M.	2009	Effects of paraquat and glyphosate on steroidogenesis in gonads of the frog <i>Rana esculenta in vitro</i> Pesticide Biochemistry and Physiology 93, 91-95 GLP: N, published: Y 2310038 / ASB2012-12007	N	MOD
KIIA 5.10 (OECD)	Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., Seralini, G.E.	2005	Differential effects of glyphosate and roundup on human placental cells and aromatase Environmental Health Perspectives 113, 716-720 GLP: N, published: Y 2310042 / ASB2009-9024 / TOX2005-1743	N	LIT
KIIA 5.10 (OECD)	Rodloff, A. C.; Krüger, M.;	2012	Chronic <i>Clostridium botulinum</i> infections in farmers Anaerobe 18 (2012) 226-228 ASB2013-13311	N	---
KIIA 5.10 (OECD)	Romano, M., Romano, R., Santos, L., Wisniewski, P., Campos, D., de Souza, P., Viau, P., Bernardi, M., Nunes, M., de Oliveira, C.	2012	Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression Archives of Toxicology 86, 663-673 GLP: N, published: Y 2310048 / ASB2012-12011	N	LIT
KIIA 5.10 (OECD)	Romano, R.M., Romano, M.A., Bernardi, M.M., Furtado, P.V., Oliveira, C.A.	2010	Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology Arch Toxicol 84, 309317 GLP: N, published: Y 2310050 / ASB2012-12012	N	LIT
KIIA 5.10 (OECD)	[REDACTED]	1987	The subacute oral toxicity of the isopropylamine salt of glyphosate (MON 0139) in female cattle 82002 ! VT-82-003 TOX9552424	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	[REDACTED]	1987	The subacute toxicity of Roundup herbicide (MON-2139) in female cattle 82001 ! VT-82-002 ASB2010-8131	N	---
KIIA 5.10 (OECD)	[REDACTED]	1987	The acute toxicity of glyphosate in female goats 80006 ! VT-80-450 TOX9552422	N	---
KIIA 5.10 (OECD)	[REDACTED]	1987	The acute oral toxicity of the isopropylamine salt of glyphosate (MON 0139) in female goats 80007 ! VT-80-451 TOX9552423	N	---
KIIA 5.10 (OECD)	Saltmiras, D., Bus, J.S., Spanogle, T., Hauswirth, J., Tobia, A., Hill, S.	2011	Letter to the Editor Regarding the Article by Paganeli et al. Chemical Research in Toxicology 24, 607-608 GLP: N, published: Y 2310056 / ASB2012-12015	N	LIT
KIIA 5.10 (OECD)	Saltmiras, D.A., Tobia, A.	2012	No Evidence of Endocrine Disruption by Glyphosate in Hershberger and Uterotrophic Assays. Abstract PS 2198 The Toxicologist (supplement to Toxicological Sciences) 126, 474 GLP: N, published: Y 2310058 / ASB2012-12016	N	LIT
KIIA 5.10 (OECD)	Savitz, D.A., Arbuckle, T., Kaczor, D., Curtis, K.M.	1997	Male pesticide exposure and pregnancy outcome American Journal of Epidemiology 146, 1025-1036 GLP: N, published: Y 2310070 / ASB2012-12022	N	LIT
KIIA 5.10 (OECD)	Shehata, A. A.; Schrödl, W.; Aldin, A. A.; Hafez, H. M.; Krüger, M.;	2012	The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro Curr Microbiol, published online 09.12.2012 ASB2012-16301	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	Shehata, A.; Schrödl, W.; Neuhaus, J.; Krüger, M.;	2012	Antagonistic effect of different bacteria on <i>Clostridium botulinum</i> types A, B, D and E <i>in vitro</i> Downloaded from veterinaryrecord.bmj.com on December 19, 2012 ASB2013-8529	N	---
KIIA 5.10 (OECD)	Song, H. Y.; Kim, J. H.; Seok, S. J.; Gil, H. W.; Hong, S. Y.;	2012	<i>In vitro</i> cytotoxic effect of glyphosate mixture containing surfactants J Korean Med Sci 2012; 27: 711-715 ASB2013-10531	N	---
KIIA 5.10 (OECD)	Takahashi, H.; Kakinuma, Y.	1992	Ammonium salt of glyphosate (MON 8750) general pharmacology study IET 90-0149/ET-92-15 TOX9552421	N	---
KIIA 5.10 (OECD)	Thongprakaisan g, S.; Thiantanawat, A.; Rangkadilok, N.; Suriyo, T.; Satayavivad, J.;	2013	Glyphosate induces human breast cancer cells growth via estrogen receptors Food and Chemical Toxicology, 59(2013)129–136 ASB2013-11991	N	---
KIIA 5.10 (OECD)	Walsh, L.P., McCormick, C., Martin, C., Stocco, D.M.	2000	Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression Environmental Health Perspectives 108, 769- 776 GLP: N, published: Y 2310118 / ASB2012-12046	N	LIT
KIIA 5.10 (OECD)	Williams, [REDACTED] [REDACTED] E. DeSesso, J.M.	2012	Developmental and Reproductive Outcomes in Humans and Animals After Glyphosate Exposure: A Critical Analysis Journal of Toxicology and Environmental Health, Part B 15, 39-96 GLP: N, published: Y 2310130 / ASB2012-12052	N	LIT
KIIA 5.10 (OECD)	[REDACTED]	1996	Glyphosate Technical: Pharmacology Screening Study in the Rat 434/021 NUF GLP: Y, published: N 2310134 / ASB2012-12054	Y	NUF

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIA 5.10 (OECD)	Yousef, M.I., Salem, M.H., Ibrahim, H.Z., Helmi, S., Seehy, M.A., Bertheussen, K.	1995	Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 30, 513-534 GLP: N, published: Y 2310142 / ASB2012-12058	N	LIT
KIIIA1 7.1.1 (OECD)	[REDACTED]	1991	Acute Oral Toxicity Study In Rats BD-91-261 MON GLP: Y, published: N 2315976 / TOX9552438	N	MOD
KIIIA1 7.1.2 (OECD)	[REDACTED]	1991	Acute Dermal Toxicity Study In Rats BD-91-262 MON GLP: Y, published: N 2315978 / TOX9552439	N	MOD
KIIIA1 7.1.3 (OECD)	Polveche, V . Rombaut, M. Bonicelli, B.	1999	Measurements of granulometry and distribution of a spray nozzle - Comparison of different glyphosate formulations 106/Pulv MON GLP: N, published: N 2315980 / ASB2012-12069	N	MOD
KIIIA1 7.1.3 (OECD)	[REDACTED]	1982	Acute inhalation toxicity of Roundup formulation to male and female Sprague- Dawley rats - incl. Amendment No. 1, Date: 15.12.1982 810093 ! ML-81-201 TOX2002-693	N	---
KIIIA1 7.1.4 (OECD)	[REDACTED] L.	1990	Primary dermal irritation study in rabbits BD-91-263 MON GLP: Y, published: N 2315983 / TOX9552440	N	MOD
KIIIA1 7.1.5 (OECD)	[REDACTED]	1991	Primary eye irritation study in rabbits BD-91-60 MON GLP: Y, published: N 2315985 / TOX9552441	N	MOD
KIIIA1 7.1.6 (OECD)	[REDACTED]	1987	Genamin T-200 BM: A closed-patch repeated insult dermal sensitization study in guinea pigs - (Buehler method) 6816-86 ! BD-86-290 ASB2010-366	N	---
KIIIA1 7.1.6 (OECD)	Griffon, B.	2001	Skin sensitization test in guinea pigs (Modified Buehler test: 9 applications) CI-2001-153 MON GLP: Y, published: N 2315987 / TOX2005-1135	N	MOD

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIIA1 7.4 (OECD)	Martin, S.; Westphal, D.; Erdtmann- Vourliotis, M.; Dechet, F.; Schulze- Rosario, C.; Stauber, F.; Wicke, H.; Chester, G.	2008	Guidance for exposure and risk evaluation for bystanders and residents exposed to plant protection products during and after application 2008/1070089 ! 1661-5751/00/000001-10 ! DOI 10.1007/s00003-008-0361-5 ASB2009-450	N	---
KIIIA1 7.5 (OECD)	Krebs, B.; Maasfeld, W.; Schrader, J.; Wolf, R.; Hoer- nicke, E.; Nol- ting, H. G.; Backhaus, G. F.; Westphal, D.	2000	Uniform Principles for safeguarding the health of workers re-entering crop-growing areas after application of plant protection product TOX2004-1971	N	---
KIIIA1 7.6.2 (OECD)	Davies, D.J.	2003	Glyphosate SL (360 g/L) Formulation (A12798Q): <i>in-vitro</i> absorption through human epidermis CTL JV1732 SYN GLP: Y, published: N 2309514 / ASB2012-11518	Y	SYN
KIIIA1 7.6.2 (OECD)	Hadfield, N.	2011	Glyphosate 360 IPA Salt (CA2273): <i>In Vitro</i> Absorption through Human Epidermis using [¹⁴ C]-glyphosate JV2147-REG NUF GLP: Y, published: N 2309512 / ASB2012-11517	Y	NUF
KIIIA1 7.6.2 (OECD)	EFSA	2012	Panel on Plant Protection Products and their Residues (PPR). Guidance on Dermal Absorption. EFSA Journal (2012), 10(4), 2665-2695, ASB2012-6959	N	---
KIIIA1 7.6.2 (OECD)	Franz, T.J.	1983	Evaluation of the percutaneous absorption of Roundup formulations in man using an <i>in-vitro</i> technique MON GLP: N, published: N 2309488 / TOX9552417	N	MON
KIIIA1 7.6.2 (OECD)	OECD	2011	Guidance notes on dermal absorption. Adopted 18 August 2011. Series on Testing and Assessment, No. 156. ENV/JM/MONO(2011)36, JT03305971. ASB2013-2 http://www.oecd.org/dataoecd/63/12/48532204.pdf .	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIIA1 7.6.2 (OECD)	Ward, R.J.	2010	360 g/L Glyphosate SL Formulation (MON 52276) - <i>In vitro</i> absorption of glyphosate through human epidermis JV2084-REG MON GLP: Y, published: N 2315989 / ASB2012-5383	Y	MOD
KIIIA1 7.6.2 (OECD)	Ward	2010	450 g/L Glyphosate SL Formulation (MON 79545) - <i>In vitro</i> absorption of glyphosate through human epidermis JV2083-REG MON GLP: Y, published: N 2309508 / ASB2012-11515	Y	MON
KIIIA1 7.6.2 (OECD)	Ward	2010	480 g/L Glyphosate SL Formulation (MON 79351) - <i>In vitro</i> absorption of glyphosate through human epidermis JV2085-REG MON GLP: Y, published: N 2309510 / ASB2012-11516	Y	MON
KIIIA1 7.6.2 (OECD)	Wester, R. C.; Melendres, J.; Sarason, R.; McMaster, J.; Maibach, H. I.	1991	Glyphosate skin binding, absorption, residual tissue distribution and skin decontamination TOX9552418	N	---
KIIIA1 7.6.2 (OECD)	Wester, R.C., Quan, D., Maibach, H.I., Wester, R.M.	2005	Percutaneous Absorption of Hazardous Chemicals from Fabric into and Through Human Skin. In Percutaneous Absorption: Drugs, Cosmetics, Mechanisms, Methods Boca Raton, FL. Taylor and Francis Group, LLC. 22, 303-310 GLP: N, published: Y 2310126 / ASB2012-12050	N	LIT
KIIIA1 7.6.3 (OECD)	EPA	2009	Alkyl Amine Polyalkoxylates; Exemption from the requirement of a tolerance Fed. Reg. 74(2009)115:28616 ASB2009-9022	N	---
KIIIA1 7.6.3 (OECD)		1973	G-3780: 14-week oral subacute study in dogs 33372 ! MRD-165 ! XX-95-336 ! MON 0818 ASB2009-9026	N	---
KIIIA1 7.6.3 (OECD)	Hahn, A.; Be- gemann, K.; Burger, R. Hillebrand, J, Meyer, H. Preußner, K. Gessener, M	2007	Ärztliche Mitteilungen bei Vergiftungen 2007, BfR ASB2013-4034	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIIA1 7.6.3 (OECD)	Holson, J.F.	1989	A dose range-finding developmental toxicity study of MON 0818 in rats. WIL Research Labs., Ashland, Ohio, USA, on behalf of Monsanto. Project no. WIL-50042, Sponsor no. WI-88-304, unpublished; ASB2009-9028	N	---
KIIIA1 7.6.3 (OECD)	[REDACTED]	1990	A developmental toxicity study of MON 0818 in rats, Final report: WI-89-388 GLP: N, published: Y 2309808 / ASB2009-9029	N	LIT
KIIIA1 7.6.3 (OECD)	Jauhiainen, A.; Räsänen, K.; Sarantila, R.; Nuutinen, J.; Kangas, J.	1991	Occupational exposure of forest workers to glyphosate during brush saw spraying work American Industrial Hygiene Association Journal, 52(1991)2:61-64 MET9600092	N	---
KIIIA1 7.6.3 (OECD)	[REDACTED]	2007	A Reproduction/Developmental Toxicity Screening Study of MON 0818 in Rats WIL-50282 GLP: Y, published: N 2309858 / ASB2010-365	Y	MOD
KIIIA1 7.6.3 (OECD)	[REDACTED]	2008	A Combined 28-Day Repeated Dose Oral (Dietary) Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of MON 8109 and MON 0818 in Rats WIL-50337 MON GLP: N, published: N 2309861 / ASB2010-364	N	MOD
KIIIA1 7.6.3 (OECD)	Martinez, T. T.; Long, W. C.; Hiller, R.	1990	Comparison of the toxicology of the herbicide Roundup by oral and pulmonary routes of exposure Z44833	N	---
KIIIA1 7.6.3 (OECD)	Stella, J., Ryan, M.	2004	Glyphosate herbicide formulation: a potentially lethal ingestion Emerg Med Australas 16, 235-239 GLP: N, published: Y 2310102 / ASB2012-12038	N	LIT
KIIIA1 7.6.3 (OECD)	[REDACTED]	1990	Ninety-day study of MON 0818 administered in feed to albino rats MSL-10468 ! ML-89-359/EHL 89161 ASB2009-9027	N	---
KIIIA1 7.6.3 (OECD)	Tai, T.; Yamashita, M.; Wakimori, H.	1990	Hemodynamic effects of Roundup, glyphosate and surfactant in dogs The Japanese Journal of Toxicology, 3, 63-68. TOX9552419	N	---

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner ⁵
KIIIA1 7.6.3 (OECD)		1991	Subchronic toxicity study in rats with Atmer 163. Hazleton Washington, Inc., Vienna, Virginia, USA, on behalf of ICI Americas, submitted by Monsanto. HWA 564-162 ASB2009-10488	N	---

Studies marked in yellow are not part of the dossier for renewal.

Codes of owner

ALK	Alkaloida Europe
ALS	Alschu-Chemie GmbH
CHE	Cheminova A/S
EGT	European Glyphosate Task Force AIR 2
EXC	ExxonMobile Chemical Belgium
FSG	Feinchemie Schwebda GmbH
HAG	Handelsgesellschaft für Baustoffe mbH & Co. KG
HEL	Helm AG
JCC	Jiangsu Changlong
LIT	Published literature
MAH	Makhteshim-AGAN Group
MOD	Monsanto Europe S.A./N.V.
MON	Montedison (Deutschland) Chemie Handels GmbH
NUF	Nufarm GmbH & Co. KG
SYN	Syntana Handelsgesellschaft